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OBSERVATIONS ON HEMOLYSINS IN ACUTE HEMOLYTIC ANEMIA

A CASE REPORT

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THE classical clinical and laboratory findings of the congenital and acquired forms of hemolytic anemia are familiar to most physicians. Occasionally, however, the clinician is confronted with patients having the findings of an acute hemolytic process for which there is no evident cause. Dameshek and Schwartz[1] critically reviewed the literature on this phase of the problem and presented their own experimental and clinical data which gave a certain amount of clarity to the subject. They postulated that hemolytic syndromes are due to hemolysins, possibly of different types and present in different amounts, functioning slowly in some cases and violently in others. Thus varying clinical and laboratory findings may be produced which may be basically related and differ only in the type and concentration of the hemolysin present.

In another paper[2] Dameshek and Schwartz listed the following five types of hemolysins which have been found in various hemolytic syndromes: two hemolysins occurring in paroxysmal hemoglobinuria, one activated by cold and one being heat stable and independent of complement action; two occurring in paroxysmal nocturnal hemoglobinuria, one heat labile and independent of complement and one an auto- and isohemolysin; and the heat labile, complement-dependent immune hemolysin found in acute nonfamilial hemolytic anemia.

In many cases, however, the nature of the hemolysin presumably present is obscure. The following case report is of interest because it illustrates some of the difficulties one may encounter in the investigation and recognition of the hemolytic factor.

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CASE REPORT

A 4½-year-old white female* was admitted to the pediatric service of the Research and Educational Hospitals for the first time with the complaints of postnasal discharge of three years' duration, generalized body pains of three months' duration, pallor and anorexia of three weeks' duration, and nausea and vomiting of five days' duration.

The parents stated that the child was well except for a slight morning postnasal discharge until three months before admission, at which time she began to complain of vague pains occurring over the entire body. This symptom was ignored by the patient's parents. Three weeks prior to entry the parents noted extreme pallor concomitant with loss of appetite. She also complained of epigastric pain occurring immediately after eating and would occasionally vomit following a meal. Five days prior to entry she complained of extreme nausea and began to vomit regularly after meals and occasionally during the night. The vomitus was watery, colorless, nonbloody, and nonprojectile. These symptoms persisted until entry, and the child lost five pounds in two weeks. Bowel habit, which had previously been normal, became irregular two days previous to entry, and an enema given resulted in only one small hard stool. She had not had symptoms referable to an upper respiratory infection and had had no respiratory genitourinary, or cardiovascular symptomatology. She was unable to retain any food for twelve hours prior to entry.

Past history revealed that the child had a normal delivery, weighed 7½ pounds at birth, and had no neonatal difficulties. At 3 weeks of age she developed a severe generalized eczema which was not controlled until she was 2 years of age. At 6 months of age, in order to attempt to control the eczema, she was weaned, and various foods were tried such as evaporated cow's milk, evaporated goat's milk, and finally a milk-free diet consisting of soybean preparations. She showed severe allergic reaction to orange juice, tomato juice, oatmeal, wheat products, and eggs. Whenever given these foods, or whenever she even touched wheat flour, her eyes, lips, and tongue would become edematous, and she would break out in generalized urticaria, indicating at the same time the presence of epigastric pain. Cod-liver oil and cevitamic acid were given at the age of 6 months. At 1 year she had a severe generalized furunculosis and again at 2 years a severe generalized furunculosis accompanied by impetigo. At 2½ years of age she had extensive swelling of the entire face, neck, and cervical glands which required lancing of one submental and one posterior cervical gland. The type of infection is not known, but it was stated that three blood transfusions were necessary. These transfusions were given approximately two years prior to this admission.

The child sat up at 9 months of age, had her first tooth at 9 months, talked and walked at 2 years. She had no contagious diseases or immunization.

The family history revealed that she was the third in a family of four children. She had one brother, now 2 years of age, who had an allergic eczema on the basis of egg protein which lasted for but nine months. There was no history of jaundice or anemia in the family.

Physical examination on admission revealed a well-developed, well-nourished, extremely jaundiced, pale, acutely ill white child. She appeared apathetic but was cooperative, intelligent, and responsive. Her skin was dry and covered with numerous generalized small macular indurated scarred areas. Her mouth showed fissuring present at the corners, somewhat similar to rhagades, her teeth were markedly carious, the lips and tongue were dry, the tonsils were enlarged, and the pharynx was slightly red. The submaxillary, tonsillar, and anterior cervical

*This patient was studied through the courtesy of Dr. Gerald M. Cline of Bloomington, Ill.

glands were all enlarged and palpable. The chest was normally elliptical in shape, and there was sight dullness to percussion in both bases and crepitant râles throughout. The heart seemed slightly enlarged to percussion in all diameters with sounds of poor quality and a soft blowing systolic apical murmur. The abdomen was rounded with poor muscle tone. The spleen was palpable 2½ cm. from the costal margin, and the liver was down one to two fingers below the costal margin in the nipple line. Examination of the extremities and nervous system was negative. The child had no rash or eruption and had not received sulfonamide drugs or other medication prior to entrance.

Laboratory tests revealed Mantoux 1:10,000 negative; admission hemoglobin was 3.3 Gm., red blood cells, 970,000; white blood cells, 16,800, with 25 per cent polymorphonuclears (13 stab forms), 56 per cent lymphocytes, and 3 per cent monocytes. The urine was positive for urobilinogen and negative for bilirubin. Corrected sedimentation rate was 12 mm. per hour; packed cell volume, 12; icterus index, 85 units; mean corpuscular volume, 121 cubic microns; mean corpuscular hemoglobin, 35 micromicrograms; mean corpuscular hemoglobin concentration, 30 per cent; nonprotein nitrogen, 33 mg. per cent; uric acid, 5.84 mg. per cent phosphatase, 6.7 units (Bodansky), carbon dioxide combining power, 47 vol. per cent; cholesterol, 157 mg. per cent; chlorides, 548 mg. per cent; calcium, 7.9 mg. per cent; phosphorus, 4.95 mg. per cent; serum albumin, 4.7 per cent; serum globulin, 1.2 per cent; bilirubin, 5.3 mg. per cent. The van den Bergh test showed an immediate color change, maximal in five minutes.

Hematologic findings showed adequate platelets and 90 per cent reticulocytes, and the bleeding and coagulation times were within normal limits. Sternal marrow aspiration demonstrated a marked normoblastic and pronormoblastic marrow. Fragility tests revealed hemolysis beginning at 0.44 per cent and complete at 0.40 per cent on several occasions.

The electrocardiogram was normal. X-rays of the colon and extremities showed no pathology. The cardiac shadow was large, and the shape was somewhat suggestive of a mitral valvular lesion; there was some evidence of soft densities in the lung fields.

From the time of admission to the hospital until eighteen days later, the child, being of group 0, received 1,750 c.c. of blood from donors of the same blood group. On the eighteenth day she had a severe urticarial reaction following a transfusion of 350 c.c. of blood. Following this, her red blood count fell from 3,130,000 to 2,250,000. Nine days later, after receiving 200 c.c. of typed and cross-matched blood, the patient had severe chills and abdominal pain, and shortly thereafter showed a marked hemoglobinuria. She had an oliguria for several days after that. At this time the patient's blood was found to be Rh negative and showed the presence of anti-Rh agglutinins. It was found that the blood which she received prior to the severe hemolytic reaction was Rh positive and that many of her previous transfusions, including her first two in this hospital, had been with Rh-positive blood.

The child was then placed on a hypoallergic diet consisting of boiled evaporated milk, carrots, jello and gelatin, rice, and potatoes. Four days later she was given 1 c.c. of egg white by mouth and immediately had a severe reaction, became cyanotic and edematous, and vomited. She responded to nasal oxygen and adrenalin, but later had generalized urticaria, vomited numerous times, and took food poorly. Her leucocyte count on this occasion rose to 33,000, and in the course of the next four days her hemoglobin fell to 5.5 Gm., her erythrocytes to 1,500,000, after having been 6.7 Gm. and 2,000,000, respectively (see Table III).

During the course of her stay in the hospital various attempts were made to affect the fragility of the red cells by the addition of certain allergens such as 0.1 c.c. egg extract, 0.2 c.c. cocoa extract, and 0.1 c.c. kidney bean extract. These had no effect upon the fragility of the red cells. Then a series was set

TABLE I

SUMMARY OF FRAGILITY TESTS UNDER VARIOUS CONDITIONS

	CONDITION OF TEST	FRAGILITY TEST RESULTS	
		HEMOLYSIS	
		STARTS PER CENT	COMPLETE PER CENT
1	Control (2 c.c. saline dilution and 1 drop patient's fresh whole blood)	0.44	0.40
2	Control 2	0.40	0.30
3	Control 3 (Done same time and temperature as Nos. 4, 5, 6, 7)	0.48	0.44
4*	0.1 c.c. egg white extract + 2 c.c. saline dilution + 1 drop whole blood	0.48	0.44
5	0.2 c.c. cocoa extract + c.c. saline + 1 drop whole blood	0.50	0.48
6	0.1 c.c. kidney bean extract + 2 c.c. saline + 1 drop whole blood	0.50	0.48
7	0.1 c.c. cocoa syrup + 2 c.c. saline + 1 drop whole blood	0.40	0.30
8	0.2 c.c. 1:10,000 histamine + 2 c.c. saline + 1 drop whole blood	0.50	0.48
9	Control 4 (same time and temperature as Nos. 8 and 10)	0.50	0.44
10	0.2 c.c. 1:10,000 histamine + 2 c.c. saline + 1 drop normal cells	0.44	0.40
11	Control 5 (just prior to hemolytic reaction No. 2)	0.44	0.30
12	Control 6 (3 hours after hemolytic reaction No. 2)	0.44	0.30
13	Control 6 (48 hours after hemolytic reaction No. 2)	0.44	0.30

*Extracts used were those found on standard testing boards.

TABLE II

SUMMARY OF BLOOD TRANSFUSIONS

DATE GIVEN	AMOUNT (C.C.)	DAYS AFTER FIRST TRANSFUSION	DONOR RH TYPE	REACTION
10/25/41	300	0	Positive	None
10/26/41	150	1	Positive	None
10/30/41	250	5	Positive	None
11/5/41	450	11	?	None
11/8/41	500	14	?	None
11/11/41	100	17	?	Urticaria
11/16/41	350	22	Positive	None
11/20/41	125	26	Positive	Chill, fever 104.2°, cyanosis, oliguria, hemoglobinuria
11/29/41	500	35	Negative	None
12/10/41	450	46	Negative	None
12/11/41	250	47	Negative	None
12/12/41	250	48	Negative	None
12/17/41	500	53	Negative	None
12/24/41	125	60	Negative	Abdominal pain, chills, fever, hemoglobinuria, hematuria
12/29/41	250	65	Pooled plasma	None

2,100 c.c. blood given prior to first hemolytic reaction.
1,950 c.c. blood given without reaction after Rh typing.

Total number of donors typed = 68.
Number of donors Rh positive = 54 (79 per cent).
Number of donors Rh negative = 14 (21 per cent).

up in which 2 c.c. of saline, 0.2 c.c. 1:10,000 histamine in the patient's blood was compared with the patient's control. Only very slight effect on the fragility was noted, and this was not thought to be sufficient to explain the child's hemolytic episodes. (Table I). In retrospect, it would have been desirable to have added complement to this hemolytic system, but unfortunately the omission was detected too late to repeat the tests.

Between Nov. 24, 1941, and Dec. 24, 1941, the child received 1,950 c.c. of Rh-negative blood without incident. On Dec. 24, 1941, during an attempt to transfuse with Rh-negative blood, it was found that after 125 c.c. of blood had been given, the child became pale and lethargic, developed severe chills, and her temperature rose to 105° F. in one hour. Two to three hours afterward she had a definite hemoglobinuria. Pre- and posttransfusion tests of the fragility revealed no change by this hemolytic reaction. Analysis of this reaction revealed that this donor had never been used before; that he had been in a fasting state from 5 A.M. until 11:30 A.M. when he gave the blood, which had then been kept in the icebox approximately twenty-four hours and had not been unnecessarily near the ice coils and had not been frozen. The blood was given approximately forty minutes after removal from the icebox and apparently was at room temperature at that time. It had not been heated. It was given at thirty to thirty-five drops per minute.

Since the hemoglobin had failed to rise with repeated transfusion, a splenectomy was performed by Dr. Warren Cole on Dec. 29, 1941. Postoperatively she ran a low-grade fever but was otherwise well until Jan. 13, 1942, when she began to have a markedly decreased urinary output. A urea clearance test was attempted, but the child put out only 3½ c.c. of urine in one hour. On Jan. 14, 1942, she began to complain of generalized abdominal pain, her temperature rose to 101.8° F., and her pulse became more rapid, but she presented no other outstanding physical findings. On Jan. 15, 1942, the patient became more pale and more jaundiced and listless and about 12 A.M. passed a good sized tarry stool that was positive for blood.

Investigation of her blood findings at this time revealed an icterus index of 25 units, hemoglobin, 2.8 Gm.; red blood cells, 1,700,000; white blood cells, 120,000; packed cell volume, 11 per cent with corrected sedimentation rate of more than 20 mm. per hour. A differential count of the peripheral blood disclosed 1 per cent basophiles, 24 per cent lymphocytes, and 75 per cent polymorphonuclears. The child became rapidly worse, and in spite of stimulants and serum therapy, expired at noon Jan. 15, 1942. Post-mortem examination revealed a well-organized noninflammatory thrombosis of the portal vein and its tributaries, parenchymatous degeneration of the heart, hemosiderosis and fatty degeneration of the liver, generalized nonspecific lymphadenopathy, anemia, and edema of the mesentery. The spleen (surgical specimen) weighed 173 Gm. and measured 14 × 6 × 4 cm. The capsule was smooth and shiny and was a uniform dark red color with a bluish gray tint. On one side were the attachments of the peritoneum which contained the vessels of the spleen and some fatty tissue. The surface had upon it many interlacing collapsed venous channels. On cut section the surface was a smooth uniform dark red in color with numerous minute grayish yellow specks scattered throughout. The consistency of the entire spleen was a uniform rubbery softness. Microscopic examination revealed that the splenic capsule was of average thickness. The trabeculae were not prominent and were absent from many areas. The splenic follicles were moderately increased in number with some secondary follicle formation. These lymphoid accumulations were generally small, with pale germinal centers composed of reticulum cells. Fibrosis of the follicle centers, the pulp, and thickening and some obliteration of the splenic arteries was a prominent feature. The most unusual feature was the enormous deposition of finely granular, refractile,

TABLE III

SUMMARY OF BLOOD FINDINGS

DATE	HB. (GM.)	R. B. C.	W. B. C.	P. C. V.* (%)	M. C. V.†	M. C. H.‡	M. C. H. C.§	CORR. SED. RATE	WELTMANN (TUBE)	ICTERUS INDEX	REMARKS
1941											
10/25	3.5	990,000	16,000	12.0	121	35.0	30.0	9		75	
10/29	7.0	3.05 M	11,500	25.5	124	36.0	29.0	0		25	
11/5	8.0	2.24	10,650	18.0	92	35.0	33.0	20			
11/10	9.5	3.13	8,750	32.0	150	44.0	29.0	1		20	
11/11											Urticaria
11/19	6.75	2.25	10,400	26.0	111	30.0	25.9	18	8.5	20	
11/20											Hemolysis
11/21	6.7	2.0	12,600	19.0	95	33.7	35.0			25	
11/24											Anaphylaxis
11/24		1.95	33,000	26.0	133						
11/28	5.5	1.5	11,100	22.0	146	36.6	25.0				
12/4	7.6	2.25	9,000								
12/8	7.1	2.6	22,500								
12/10	7.0	1.8	11,400	22.0	122	38.0	32.0	18			
12/12	8.0	2.2	12,500								
12/14	7.0	1.9	9,600								
12/18	6.7	2.5	11,600	25.0	100	26.0	26.0	10		18	
12/20	6.6	2.1	14,600								
12/23	6.0	1.9	11,400	15.0	126	31.0	40.0	9		22	
12/23											Hemolysis
12/24	5.2	1.5	15,400	15.0	100	34.0	34.0	8		100	
12/27		1.5	11,400	18.0	120			11		35	
12/29											Splenectomy
12/30	7.5	2.86	16,550	29.0	102	26.0	26.0	0			
12/31	7.5	2.65		28.0	105	28.0	26.0	0		8	
1942											
1/1	8.0	2.4		29.0	121	33+	27+	4			
1/2	8.8	2.5		30.0	120	35	29	0			
1/3	8.8	2.62		30.0	114	33	29	0			
1/4	8.8	2.71		30.0	110	32	29				
1/5	8.8	2.65		30.0	113	33	29	0			
1/7				33.0				10			
1/12				24.0				6			
1/14	6.5	2.2	14,200				29				Blood in stool
1/15											
1/15	2.6	1.17	120,000	11.5	98	22	22	20+	1	25	Expired
1/15											

*P.C.V., Per cent of corpuscular volume.
†M.C.V., Mean corpuscular volume.
‡M.C.H., Mean corpuscular hemoglobin.
§M.C.H.C., Mean corpuscular hemoglobin concentration.

orange-yellow crystalline bodies throughout the whole spleen. These probably represented cholesterol from hemolyzed blood, of which there was considerable throughout.

DISCUSSION

The discussion of the clinical and experimental findings in this case is concerned with an attempt to explain the nature of the patient's hemolytic reactions.

Table I summarizes the results of the tests of the fragility of the patient's cells in contact with extracts of allergens to which the patient had a known sensitivity and in contact with varying dilutions of histamine. It will be seen that little or no effect upon cell fragility could be demonstrated by means of the fragility tests run under these conditions. The unfortunate omission of complement in this test has been commented upon. The case history has shown that following the anaphylactoid transfusion reaction, the child suffered red cell destruction. However, this hemolysis may have resulted from anti-Rh agglutinin activity. The anaphylaxis from orally ingested egg white was followed by a falling red count, but this occurred during a period following the severe hemolytic transfusion reaction and probably was not on an allergic basis. From these facts, limited as they are, we were unable to demonstrate definitely any etiologic relationship between the allergic state of this patient and her hemolytic episodes. That the hemolytic state was in no measure influenced by splenectomy is well evidenced by the postoperative course of the patient.

As stated in the case history, this patient suffered three reactions following transfusions, the first consisting of only a moderately severe anaphylactoid reaction with incipient hemolysis and the last two being definitely hemolytic in nature (see Table II). The first reaction resulted from the use of blood from a donor who had previously eaten egg and cereals to which the patient was sensitive. The donor had apparently misunderstood directions and had failed to state that he had eaten prior to giving the blood. It seems most likely that this reaction was simply an allergic response on the part of the patient to intravenously administered allergens; however, it may have been a premonitory symptom of the rising anti-Rh agglutinin titer in the patient's blood. The second explanation is supported by Wiener's Case 4.[3] This patient also suffered an urticarial reaction concomitant with the hemolytic transfusion reaction. The second reaction was by far the most serious, resulting in a marked hemoglobinuria and oliguria. Previously the child had received 2,100 c.c. of blood in six transfusions over a period of two and one-half weeks with no reaction other than the anaphylactoid one previously mentioned. Cross-matching by ordinary methods had revealed no cross-agglutination in either patient's or donor's serum, and the donor's blood was of the same blood group as the patient's. Following this severe reaction, Rh typing revealed that the patient was Rh negative and had anti-Rh agglutinins in her

†M.C.V., Mean corpuscular volume.
‡M.C.H.C., Mean corpuscular hemoglobin concentration.

serum and that the donor's blood was Rh positive. Further study showed that father and mother, who had given the first and second transfusions, and the donor immediately preceding the reacting donor, were all also Rh positive.

The Rh factor was discovered recently by Landsteiner and Wiener[4] and was found by Wiener and Peters,[5] and since by others, to be responsible for many hitherto unexplainable serious and fatal hemolytic blood transfusion reactions in patients of the same blood groups in whom the usual blood grouping and compatibility tests failed to reveal any evidences of incompatibility. It is assumed that the Rh factor, which is present in about 85 per cent of human beings (called Rh positive) and absent in 15 per cent (called Rh negative), has the ability when injected into Rh-negative persons to stimulate in them development of agglutinins against the Rh-positive blood. When these Rh-negative persons again receive blood with the Rh factor in it, the blood which had previously been compatible acts as incompatible and may give rise to severe reactions.

After the discovery that the patient had anti-Rh agglutinins in her serum, 1,950 c.c. of Rh-negative blood (five transfusions) were given in the course of four weeks with no reaction until the child had a moderate hemolytic reaction with hemoglobinuria after receiving 125 c.c. of Rh-negative blood.

Following this second hemolytic episode, it was found that the patient's serum also contained so-called cold agglutinins of a rather wide thermal range. Her serum clumped her own cells at icebox and room temperatures but not at body temperature. It is of some interest that her cold agglutinins manifested a certain degree of selectivity. The child's serum was tested for cold agglutinins with the bloods of twenty-six type O, Rh-negative individuals, all prospective donors. The tests were set up by placing two drops of the patient's serum and one drop of a 1 to 2 per cent suspension of the cells in narrow test tubes (7 mm. inside diameter) and incubating at about 0°, 22°, and 37° C. for one hour. The results were read with the naked eye and checked with the microscope. There was no agglutination with the blood of twenty persons, four blood specimens were clumped at 0° C. only, and two at both 0° and at 22° C. The agglutination at 22° C. was feeble. It is generally accepted that so-called cold agglutinins are not responsible for blood transfusion reactions. One of us (I. D.) is of the opinion that it is not impossible that the so-called cold agglutinins of the patient reacted with the transfused red cells. In another publication[6] by one of us (I. D.), the possible role of cold agglutinins in blood transfusion reactions will be discussed in greater detail. It is possible that the period of forty minutes during which the blood was at room temperature had not been sufficient to raise its temperature properly.

To summarize the transfusion reactions, one may have been due to allergens in the donor blood or may have been an expression of a rising titer of anti-Rh agglutinins; one was definitely due to Rh sensitization; and one could not be explained satisfactorily, unless the presence of so-called cold agglutinins is considered to be responsible.

At the bottom of Table II is indicated the relative incidence of Rh-negative and Rh-positive types in this series which agrees well with published values.

CONCLUSIONS

A case of unsuccessfully splenectomized acquired hemolytic anemia with normal fragility and marked reticulocytosis is reported with certain observations:

1. In spite of the presence of marked food allergy, attempts to correlate the actions of allergens and histamine with the hemolytic process were unsuccessful, suggesting that in this case at least no definite relationship between allergy and hemolysins could be demonstrated. Possibly the use of complement plus the specific allergens to which the patient was sensitive might have demonstrated changes in fragility.

2. Successful transfusion with Rh-negative blood was given in a patient who had previous transfusion reactions due to anti-Rh agglutinins. This suggests that in all cases of hemolytic anemia not only should both major and minor transfusion reactions be carefully studied, but as a prophylactic measure more sensitive cross-matching procedures should be employed than is routinely done. Rh transfusion reactions are apt to be more frequent in such cases because of the necessity for repeated transfusions with the concomitantly increased opportunity for isoimmunization with the Rh factor in case the patients are Rh negative.

3. Support is given to the thesis that in some cases cold agglutinins may be the cause of hemolytic transfusion reactions. For this reason, wherever possible, blood should be given at room temperature.

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PREVENTION OF RICKETS WITH SINGLE MASSIVE DOSES OF VITAMIN D

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THE use of single massive doses of vitamin D for the prevention of rickets has naturally developed out of its use for the treatment of rickets. The possibility of administering a single dose of vitamin D to an infant only two or three times a year in order to protect him against rickets not only excites the imagination but has great public health potentialities. Sufficient clinical evidence has accumulated to show that this is possible.

REVIEW OF LITERATURE

Although Vollmer[1] as early as 1928 first successfully protected rats from developing rickets by the administration of a single massive dose of vitamin D, about one thousand times the effective single daily dose, it was not until ten years later that this method of rickets prevention was tested clinically in Germany. In 1938 Harnapp[2] reported a group of seventy-one infants to each of whom he had given a single massive oral dose of 5.0, 7.5, or 10 mg. (200,000, 300,000, or 400,000 units) of vitamin D_2 or D_3 for the prophylaxis of rickets. The infants varied in age from 2 months to 1½ years. The study was started in December and ended the middle of February, an observation period of ten weeks. The group was examined monthly for clinical and roentgen evidence of rickets. Only one 6-month-old infant developed craniotabes on 5 mg. (200,000 units) of vitamin D, four weeks after it was given, and eight weeks after administration showed some irregularity of the ulnar metaphyses. No difference between the efficacy of vitamin D_2 and D_3 could be determined.

In 1938 Harnapp[3] reported another group of infants ranging up to the age of 1½ years whom he had given single massive prophylactic doses of vitamin D_2. This study was begun earlier in the winter, and only infants were included who could be observed for at least from four to six months. As in the previous study, clinical and roentgenographic studies were carried out. Thirty-six infants received 5 mg., forty-two infants 7.5 mg., and twenty-eight infants 10 mg. of vitamin D_2 (200,000, 300,000 and 400,000 units, respectively) without regard to age. None of the infants receiving 7.5 or 10 mg. of vitamin D_2 developed rickets clinically or on roentgen examination. Of the thirty-six infants who received 5 mg. of vitamin D_2 thirty-one remained free

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U. S. P. units referred to throughout.

of demonstrable rickets; two developed craniotabes which disappeared without further treatment and showed no roentgen evidence of rickets; and two infants after four months developed moderate craniotabes and mild roentgenographic evidence of rickets which disappeared following the administration of a second dose of 5 mg. of vitamin D_2. One premature infant who received 5 mg. at 2 months of age showed craniotabes and mild rickets by roentgenogram five months later. Ten milligrams of vitamin D_2 were administered, and healing was complete in one month. Harnapp concluded that a single prophylactic dose of 5 mg. (200,000 units) lies on the borderline of the necessary amount of vitamin D for this form of therapy. On this dosage the development of rickets is possible after a period of time. Therefore the single prophylactic dose should range between 7.5 and 15 mg. (300,000 and 600,000 units), or if 5 mg. is used, it should be repeated during the winter months.

Others in Germany have also studied the prophylactic effect of single massive doses of vitamin D given orally. Windorfer[4] in 1938 used a single oral dose of from 6 to 8 mg. (240,000 to 320,000 units) of vitamin D_2 in premature infants. Of thirty-two infants observed for from three to eight months, nineteen remained free of rickets, seven developed slight rickets, and six developed distinct rickets. In commenting on this report, in the light of his own experience, Harnapp recommended a minimum dosage of at least 10 mg. (400,000 units) of vitamin D_2 or D_3 for premature infants.

Broekmann[5] studied 100 infants of different ages convalescing from dystrophy but free from rickets as demonstrated clinically and serologically. They were given oral doses of 15 mg. of vitamin D_2 or D_3. Only sixty infants could be followed throughout the entire winter of 1937-1938. The infants were examined clinically and serologically at two- to four month intervals. No roentgenograms were taken. According to his observations, thirteen infants after a period of two and one-half months showed either craniotabes or a slight depression of the serum phosphorus. He could determine no difference in the efficacy of vitamin D_2 and D_3. According to his findings, he recommended that one single prophylactic dose of 600,000 units be given in October and a second one the end of January.

During the winter of 1938-39, Schwartzer[6] studied all the infants in a children's home who were given doses of either 7.5 mg. or 15 mg. (300,000 and 600,000 units) of vitamin D_2 or D_3. The vitamin was emulsified in milk. Monthly x-rays were taken. The number and ages of the infants studied were not stated. A control group was followed. During the first six months these control infants developed mild but recognizable rickets which healed later when exposed to sunlight. Those infants receiving 15 mg. doses remained free of rickets. However, one isolated case, at the end of the winter, showed an irregularity of the metaphysis, which disappeared spontaneously. Also in premature in-

fants, rickets was well prevented. Only one premature infant showed craniotabes and on roentgenogram slight rickets after three and three-fourths months which disappeared spontaneously under observation. Doses of 7.5 mg. were insufficient to prevent rickets dependably. The author saw no toxicity, osteoporosis or pathologic calcification, or abnormal ephiphyseal structure from these high doses when a pure preparation was used.

Reitschel, Horster, and Misslbeck,[7] in 1940, also reported a large group of infants who were given single massive doses of from 5 to 15 mg. of vitamin D_2 or D_3. They found that 5 mg. (200,000 units) cannot be depended upon for single massive dose prophylaxis but that from 7 to 10 mg. (280,000 to 400,000 units) is better. These authors saw no need for larger doses than the latter.

Hartenstein,[8] in 1940, studied several hundred infants with single massive doses of vitamin D in order to determine the minimum effective massive dose necessary for rickets prophylaxis and to differentiate between the efficacy of vitamins D_2 and D_3. He found that 78 per cent of infants in a control group developed rickets, whereas of 102 infants given 4.5 mg. of vitamin D_2 (180,000 units), 61.7 per cent developed rickets; of eighty-two infants given 180,000 units of vitamin D_3, only 23.8 per cent developed rickets. He therefore concluded that vitamin D_3 was superior to vitamin D_2 in a three-month follow-up of cases and that 180,000 units was an insufficient dose for massive dose rickets prophylaxis. In full-term infants (only seven infants studied), he found that 6 mg. (240,000 units) of vitamin D orally was sufficient to protect against rickets for five months. Doses of from 6 to 7.5 mg. in five premature infants were not sufficient. The vitamin is best given in milk. He pointed out that in studies to differentiate between the efficacy of vitamins D_2 and D_3 it was necessary to use minimal doses as he did.

Another prophylactic study done in Germany was reported by Heisler[9] who treated 116 infants ranging in age from 8 weeks to 20 months with oral and intramuscular doses of 5 mg., 7 mg., 10 mg., or 15 mg. of vitamin D_2 or D_3 (200,000, 300,000, 400,000 or 600,000 units). The observation period lasted from thirty to 240 days, with an average of 110 days. The infants were examined every six weeks. No blood determinations were made. The 5 mg. and 7.5 mg. doses were given in a single dose. The 10 mg. and 15 mg. doses were divided into two or three parts, respectively, and given within one week. Six infants were given the vitamin intramuscularly because of the presence of fever or an intestinal disturbance. The study was made to determine the efficacy of the smallest single mass dose necessary to protect against rickets. No previous antirachitic agent had been given. Heisler found no instance of failure if the shock dose was 10 mg. or more either in the vitamin D_2 or D_3 group. In the vitamin D_2 group of sixty infants, six of whom had received a 5 mg. (200,000 units) dose,

rickets developed. Of the fifty-six infants who received vitamin D_3, one who had been given a single prophylactic dose of 5 mg. developed rickets. The first sign of rickets in these infants developed six weeks to sixteen weeks later. Heisler's conclusions were that vitamin D_3 seemed more effective than vitamin D_2 in single massive prophylactic doses. Ten milligrams or more of vitamin D_2 or D_3 given in a single prophylactic dose will prevent rickets for the duration of the winter months. If 5 mg. of vitamin D are given, rickets may not be prevented, and a second 5 mg. dose or more is usually necessary later in the winter.

Hofmeier,[10] in the report of a public health project on the prophylaxis of rickets, prescribed daily doses of irradiated ergosterol for the average baby. Under unfavorable social conditions, or where the mother was found unwilling to cooperate, the single massive dose prophylaxis was carried out. He administered up to 15 mg. (600,000 units) of vitamin D_2 orally in a single dose and found that it was sufficient to protect the infant against rickets for from four to six months.

In this country, Zelson,[11] in 1940, reported on the prevention of rickets in forty-six premature infants with a single massive parenteral or oral dose of various vitamin D preparations. A single dose of 600,000 units of crystalline vitamin D_2 or D_3 in 0.7 c.c. of peanut oil and 0.3 c.c. of ether was administered parenterally to twenty infants. The others received a single dose of 200,000 to 500,000 units of an electrically activated form of ergosterol (ertron). The vitamin D was administered at about 2 weeks to 1 month of age. Only seventeen of these premature infants could be followed. Zelson states in his summary that they were followed up to an age of from 44 to 279 days and that during these periods no rickets appeared in the premature infants who received 600,000 units of vitamin D_2 or D_3 parenterally. One premature infant who received 500,000 units of the oral vitamin D preparation on the twenty-first day of life showed rickets at the beginning of the fourth month; that is, two and one-half months later. However, if one examines Zelson's table of cases in greater detail, one finds that only one infant who received vitamin D_2 parenterally was followed for eight and one-half months without developing rickets, whereas the other eleven infants were followed only from thirteen days to a maximum of sixty-two days, most of them only for thirty days.

REPORT OF THIS STUDY

This study was undertaken to determine the efficacy of single massive oral doses of an electrically activated ergosterol* for the prevention of rickets in young infants. The study was begun in September, 1941,

*Erton, Nutrition Research Laboratories, Chicago, Illinois. In the manufacture of Ertron, ergosterol is first heated in a partial vacuum until it is vaporized. While it is in a gaseous state, an electric current is passed through it, of controlled amperage and voltage, and for a definite length of time. In this way, all of the ergosterol is exposed to exactly the same amount of activation. It is then bioassayed according to the U. S. P. method for vitamin D and diluted in a solid fat and other inert material.

and was concluded in June, 1942. The babies that were studied were seen either in private practice or in the Matlock Street Well-Baby Station of the Paterson Board of Health, or the pediatric clinic of the Barnert Memorial Hospital, Paterson, N. J. The major part of the work was done at the Matlock Street clinic, which is supervised by a trained nurse who made frequent social service calls to the home. Many mothers brought their babies to the clinic for weighing at weekly intervals, but a clinical examination for rickets (beading, craniotabes, knobbing of wrists, and Harrison's groove) was made only once a month. Only nonirradiated evaporated milk* was used.

Inasmuch as it had become the established practice to give young infants some antirachitic agent soon after birth, it was not possible at the beginning of this study to enroll infants who had received no antirachitic agent in the form of irradiated evaporated milk or some fish oil. However, as soon as an infant was first seen he was placed on nonirradiated evaporated milk or a nonvitamin D cow's milk with Karo syrup added, and all other antirachitic substances were discontinued. At or just prior to the third month of life, the infant was started on a precooked cereal, and usually in about one week he was taking a sufficient amount in which to mix the electrically activated ergosterol. Each capsule of this preparation (ertron) contains 50,000 units of vitamin D. The mother was given twelve capsules (600,000 units) with printed as well as oral instructions for its administration. She was told to empty the contents of six of the capsules and mix same with one tablespoonful of precooked cereal. Then the warm formula was added, the mixture stirred, and fed to the baby. The second six capsules were similarly administered the following morning. The purpose of this was (1) to avoid altering the taste of the cereal, (2) in case the feeding was refused or regurgitated, only six capsules would be lost, and (3) probably better absorption with less loss of the vitamin through the bowel was possible if the activated ergosterol was given in two divided doses twenty-four hours apart.

Precooked cereal with milk is ideally suited for the administration of this preparation of activated ergosterol. However, the milk formula or other solid foods may be utilized. The activated ergosterol is well absorbed in cereal and does not alter the taste of the food. In only two or three instances was the feeding either refused or regurgitated by the infant. In these cases a second set of six capsules was repeated the following week.

A second dose of 600,000 units of electrically activated ergosterol was administered in cereal from three to six months after the first dose. A roentgenogram was taken prior to the administration of the first dose in most instances. Follow-up roentgenograms were taken from one to six months after the administration of the single massive doses of vitamin

*Sheffield's Sealect, or A & P Whitehouse brand of evaporated milk.

D. If a roentgenogram had not been taken at the onset of prophylaxis, one was taken prior to the administration of the second mass dose. All the films were read by Dr. Roemer and reviewed by both of us as a whole at the end of the study. The criteria laid down by others in the study of rickets prevention was followed.[12] Roentgenograms of both wrists were taken at each examination.

New foods were introduced into the diet in the following manner: ripe mashed banana and cooked cereals about the fourth month of life; strained vegetables about the fifth month; stewed fruits and puddings about the sixth and seventh months; egg yolk was restricted and was usually not given until the tenth month.

Seventy-five infants were given 600,000 units of electrically activated ergosterol (ertron) in two divided doses, but only sixty-two infants could be followed. Forty-three were examined roentgenologically at the onset of the study, and nineteen were not. Of these forty-three infants, fourteen were found to have slight rickets and four were found to have more marked rickets (Table I). Only those cases were considered rachitic which on subsequent roentgenograms showed definite evidence of healing of the rachitic process. Clinically, nine of these infants showed craniotabes, beading, flaring, or knobbing in decreasing order of frequency.

TABLE I

I. Total number of infants to whom 600,000 units of electrically activated ergosterol were administered			75
II. Total number of infants followed			62
A. Number of infants x-rayed at onset of study		43	
1. Number showing mild rickets	18		
a. Breast-fed—no antirachitic	4		
b. Irradiated evaporated milk	3		
c. Daily dose of antirachitic	11		
2. Number showing no rickets	25		
a. No antirachitic	8		
b. Average daily antirachitic	15		
c. 600,000 units vitamin D previous summer	2		
B. Number of infants not x-rayed at onset		19	
a. No antirachitic	5		
b. Irradiated evaporated milk	1		
c. Irradiated evaporated milk and daily antirachitic	11		
d. 600,000 units vitamin D previous summer	2		

The eighteen cases of mild rickets will be analyzed first. All of these infants were born at term, but two weighed only 5 pounds at birth. None were colored. Four were breast fed for one month or longer but received no previous antirachitic in the form of irradiated evaporated milk, fish oil, or irradiated ergosterol. Three others received irradiated evaporated milk for about one month but no other antirachitic prior to receiving 600,000 units of vitamin D. The remaining eleven infants in this group of eighteen mildly rachitic infants were on irradiated evaporated milk and average prophylactic doses of 50 per cent percomorphum oil, or viosterol for one month or more prior to receiving

A.

B.

Fig. 1.—*A*, Active rickets in a colored infant, aged 18 months, not included in this series but shown to demonstrate the manner in which calcium is deposited in a severe case of rickets following the use of a single massive dose of vitamin D.

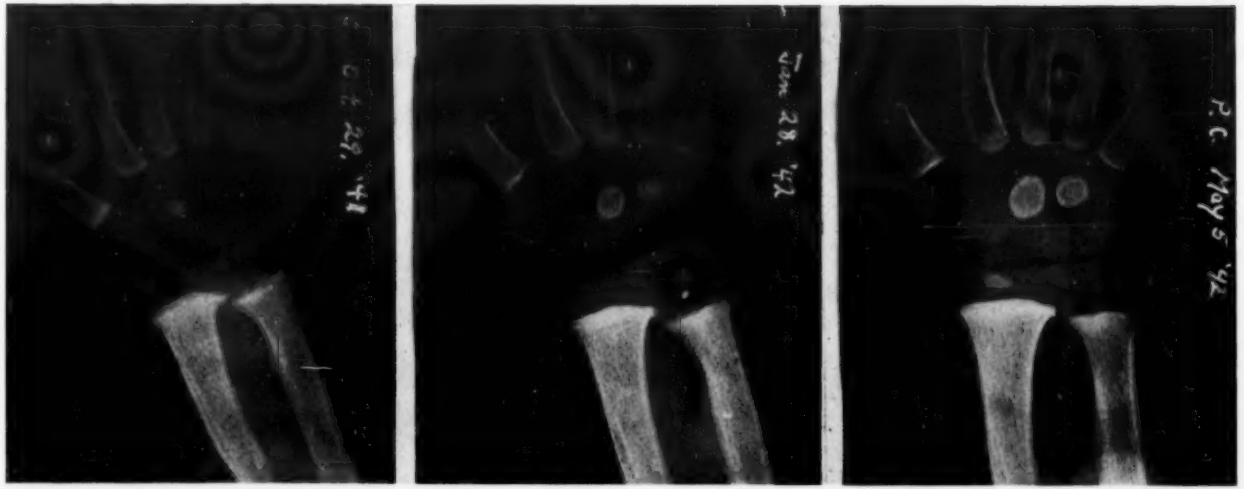
B, Three weeks after a massive dose of 600,000 units of electrically activated vitamin D—marked deposition of calcium at metaphyses. Compare with calcium deposition in mild case of rickets, Fig. 5. (From J. M. Soc. New Jersey **38**: 436, 1941.)

a single massive dose of vitamin D. Roentgenograms were first taken between the third and fifth month of life and showed mild or slight rickets. Each was then given 600,000 units of electrically activated ergosterol orally in two divided doses. Roentgenograms were not taken at regular intervals, so that the exact time of healing is not known, but this was observed one or more months later, depending on the time a follow-up roentgenogram was taken. The striking deposition of calcium as is seen in infants with severe cases of rickets treated with a single massive dose of vitamin D was not observed in these mild cases (compare Figs. 1 and 5). Eight of these infants could be followed only for one to two months, during which time the rachitic process showed partial or complete healing. Three were followed for three to three and one-half months, two were followed for from four to four and one-half months, three for from five to five and one-half months, and two for from six to seven months (see Table II). The rachitic process healed during this observation period in these cases and no further activation was seen despite the fact that only a single massive dose of vitamin D had been given two to seven months previously in ten of the cases (see Figs. 2 to 5).

TABLE II

NUMBER OF CASES FOLLOWED FOR				
1 TO 2 MO.	3 TO 3½ MO.	4 TO 4½ MO.	5 TO 5½ MO.	6 TO 7 MO.
a. Eighteen Infants Showing Mild Roentgenographic Rickets at Onset of Study; Follow-up Showed Healing of Rickets and No Recurrences				
8	3	2	3	2
NUMBER OF CASES FOLLOWED FOR				
2 TO 2½ MO.	3 TO 3½ MO.	4 TO 4½ MO.	5 TO 5½ MO.	6 TO 7 MO.
b. Twenty-five Infants Showing No Roentgenographic Evidence of Rickets at Onset and No Rickets on Follow-Up Roentgenograms				
1	5	9	9	1
c. Nineteen Infants Not Roentgenographed at Onset of Study; No Rickets on Follow-Up Roentgenograms.				
4	5	5	3	2

It was said that forty-three infants were examined roentgenologically at the onset of this study and that eighteen were found to be mildly rachitic. The remaining twenty-five infants in this group showed no rickets on first roentgenograms. These latter infants varied in age from 2 to 6 months when first observed. Fifteen had received average prophylactic amounts of vitamin D for a month or more, eight had not, and two had each received a single massive dose of electrically activated ergosterol during the previous summer months. At ages varying between 3 and 8 months, these infants were given an oral massive prophylactic dose of electrically activated ergosterol, 600,000 units, in two divided doses. At the onset of the study, although roentgenograms of the wrists were negative for rickets, four of the infants showed

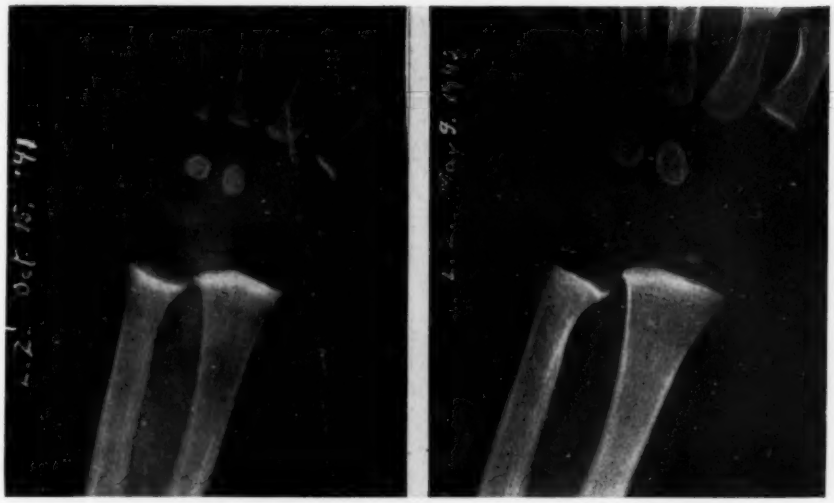


A. *B.* *C.*

Fig. 2.—P. C. *A*, Age, 3 months, 3 weeks; weight, 10 pounds, 11 ounces. Received irradiated evaporated milk and ten drops of viosterol daily for two and one-half months. Roentgenogram Oct. 29, 1941, showed mild rickets. Vitamin D (ertron), 600,000 units, given next two days in two divided doses.

B, Jan. 28, 1942, three months after massive dose of vitamin D—rickets healed. A second dose of 600,000 units repeated at this time.

C, May 5, 1942, three months and eight days after second massive dose of vitamin D—normal metaphyses at wrist. Age, 10 months; weight, 18 pounds, 9 ounces; height, 28½ inches.



A. *B.*

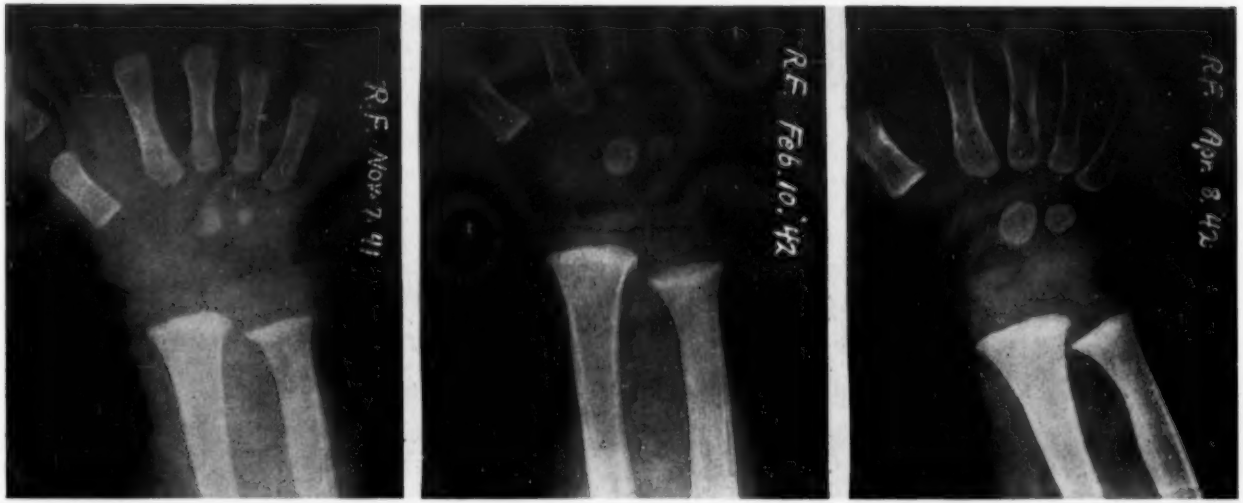
Fig. 3.—L. Z. *A*, Age, 3 months, 15 days; weight, 12 pounds, 13 ounces. Breast fed four weeks; received irradiated evaporated milk and ten drops of 50 per cent percomorphum oil daily for over two months, but took latter poorly. Roentgenogram on Oct. 15, 1941, showed moderate rickets at wrist. Given 600,000 units of ertron in two divided doses. Second dose of 300,000 units was regurgitated and was repeated seventeen days later (Nov. 9, 1941), when mother returned to clinic.

B, May 9, 1942; age, 10 months; weight, 19 pounds, 9 ounces; height, 29¼ inches. Six months after repeat dose of 300,000 units of vitamin D shows ulnar metaphysis healed, with only slight fraying at one end still present. More effective absorption and retention would have been assured if this infant had taken his 600,000 units of vitamin D in two divided doses twenty-four hours apart rather than seventeen days apart.

A. *B.*

Fig. 4.—A. P. *A*, Age, 4 months, 13 days; weight, 11 pounds. Received irradiated evaporated milk a short time but no other source of vitamin D. Roentgenogram Dec. 3, 1941, showed mild rickets. Given 600,000 units of ertron in two divided doses.

B, Five months after massive dose of vitamin D. Age, 9 months, 12 days; weight, 19 pounds; height, 26¾ inches. Roentgenogram shows some irregularity of ulnar metaphysis.

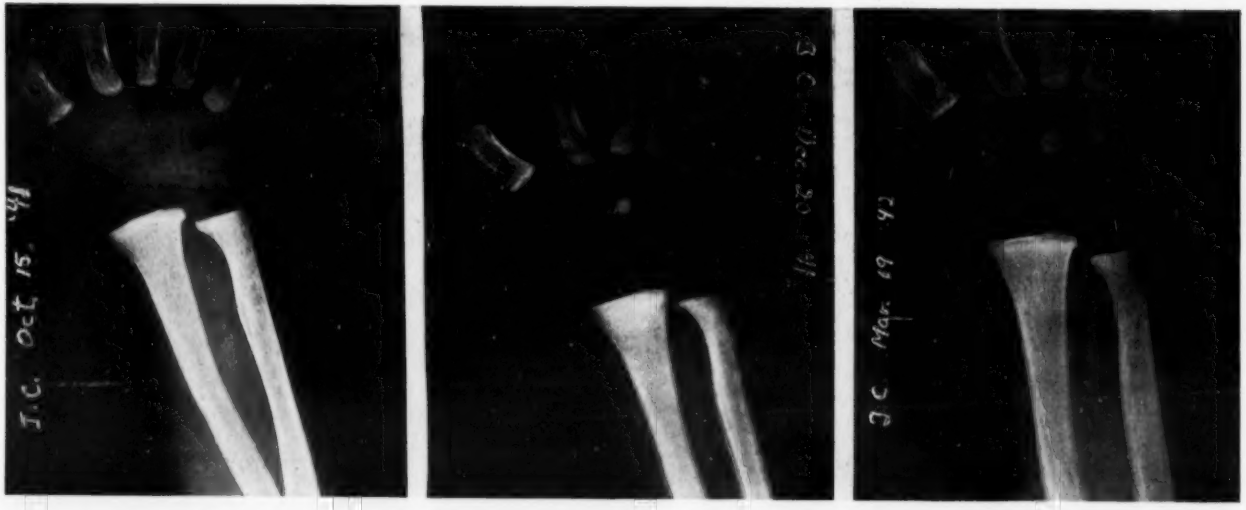


A. *B.* *C.*

Fig. 5.—R. F. *A*, Age, 3 months, 7 days; weight, 15 pounds, 12 ounces; height, 25 inches. Received irradiated evaporated milk one month and no other antirachitic agent. Roentgenogram Nov. 7, 1941, showed mild rickets. Given 600,000 units of ertron in two divided doses, twenty-four hours apart.

B, Three months and three days after massive dose of vitamin D; roentgenogram shows healing of rachitic process. Compare with marked calcium deposition in case of severe rickets in Fig. 1.

C, Five months following massive dose of vitamin D. Age, 8 months, 7 days; weight, 23 pounds; height, 29 inches; roentgenogram shows rickets healed and no reactivation.



A. *B.* *C.*

Fig. 6.—J. C. *A*, Age, 5 months; weight, 10 pounds, 4 ounces; premature at birth, weighing 4 pounds, 10 ounces. Received irradiated evaporated milk until 5 months of age and ten drops of 50 per cent percomorphum oil for four weeks. Roentgenogram Oct. 15, 1941, showed no evidence of rickets. Given 600,000 units of ertron in two divided doses.

B, Two months and five days after massive dose of vitamin D; wrists negative for rickets.

C, Five months and four days after massive dose of vitamin D; still protected against rickets. Age, 9 months, 3 weeks; weight, 19 pounds, 5 ounces; height, 28 inches.

moderate or mild craniotabes. All except two infants took the preparation well in precooked cereal. One infant refused to take cereal by the fourth month, and the activated ergosterol was administered in milk. The first day he left over one ounce of the formula containing the massive dose. The second day he regurgitated a little of the formula. Despite this, however, a follow-up roentgenogram taken three months, and again five months, after the prophylactic dose showed that he was protected from rickets. The second infant vomited the cereal feeding in part which contained the prophylactic dose. Six capsules (300,000 units) were repeated four days later. A roentgenogram three months later showed his ulnar metaphyses to be normal.

The remaining twenty-three, as well as the two others, in this group of twenty-five infants whose roentgenograms were normal when first taken, remained normal for a follow-up period of from two to six and one-half months after the single massive oral dose of vitamin D (see Table II). Two infants in this group were premature. One was protected for five months (see Fig. 6), the other three and one-half months, and then two and one-half months more, after a second massive oral dose of vitamin D. Two infants in this group were colored. One of these was followed three months and the other five and one-half months without developing rickets.

The remaining group to the considered, out of a total of sixty-two infants followed, are nineteen who were not x-rayed at the onset of the study. Of these, eleven received irradiated evaporated milk and average daily prophylactic doses of vitamin D for a period of time prior to the administration of the single massive prophylactic dose. One infant had been on irradiated evaporated milk only, and five had received no previous antirachitic agent. Two of the infants each had received a single massive dose of 600,000 units during the summer months about six months prior to a second administration during the winter. The ages at which the single oral mass prophylactic dose was given varied between three and six months. Follow-up roentgenograms were taken two to six months later and were found to be normal. Ten infants were given a second mass dose of 600,000 units from three to six months after the first one, and roentgenograms taken two to five months later again were normal (see Table II).

Well over one hundred doses of 600,000 units of electrically activated ergosterol in two divided doses have been administered to infants ranging in age between 3 months and 1½ years of age. Numerous infants were given a second dose of 600,000 units from three to five months after the first administration. The vitamin D given in this form was well tolerated and no toxic manifestations were observed. On the contrary, these infants thrived exceptionally well and many showed better than average weight gains following the administration of the single mass doses. No diminution in the incidence of respiratory infections was observed.

On single massive prophylactic doses of vitamin D administered orally (600,000 units in two divided doses), forty-four infants were followed for from two to seven months without any evidence of rickets; eighteen infants who showed mild rickets at the onset of the study showed healing of the rachitic process during the period of observation and showed no reactivation of the rickets for from two to seven months, depending on the follow-up period.

COMMENT

Advantages of Massive Dose Vitamin D Prophylaxis of Rickets.—Before entering into a discussion of the various aspects of the prophylaxis of rickets with massive doses of vitamin D, it would seem advisable to list the advantages of such a prophylactic measure. The first advantage which comes to mind is the time saving factor. Instead of administering daily doses of an antirachitic agent, the mother has to administer an oral dose only once or twice during the winter and spring months. Second, fish oils are often objectionable both to mother and baby. Infants frequently develop rickets on such a regime because they refuse or spite out the oil, even when it is given in drop doses, and no substitute is administered. Other infants regurgitate the fish oil because it disagrees with them, or on account of the presence of other food allergies. In these infants a single massive prophylactic dose is of great value because once the vitamin has been administered the infant is protected for a period of months. In the case of an allergic infant, the activated ergosterol is given during a vomiting-free period. A third advantage of this method of prophylaxis is its low cost. The infant remains protected for a period of about five months for somewhat more than it costs to protect the infant for a month or so on ordinary antirachitic agents. The massive prophylactic dose method has great importance in public health work. In clinic practice many mothers are uncooperative due to ignorance, whereas others are unable to purchase the more expensive antirachitic agents at regular intervals. As a result, many infants in this social group receive antirachitic prophylaxis at irregular intervals. For these patients the massive dose vitamin D prophylaxis of rickets is the method of choice.

Rationale of Massive Dose Vitamin D Prophylaxis of Rickets.—If single massive doses of vitamin D can protect against rickets for a period of months, it can be postulated that the vitamin is absorbed into the blood stream and stored in the tissues until depleted. There is experimental as well as clinical evidence to support this theory. It has been shown that the vitamin is absorbed unchanged and that it circulates in the blood; that it is stored in various tissues without inactivation; that it may be metabolized and excreted in amounts depending on the dose administered.[13] Light, Wilson, and Frey[13] found that a surprisingly high percentage of the vitamin D fed experimental cows was absorbed into the blood stream. Guerrant, Morck, Bechdel,

and Hilston[13] fed young calves a total intake of 100,000 to 800,000 units of vitamin D over a period of months. According to these authors, only a relatively small percentage of the antirachitic substance (cod-liver oil or irradiated yeast) was stored in the blood or the liver, using biologic tests. It should be pointed out that they used only small doses of vitamin D in relation to the weight of the animals. They do state, however, that those animals which received the greatest daily intake of vitamin D also showed the greatest amount in their tissues.

Experimental work in animals and human beings, utilizing massive doses of vitamin D, shows that the vitamin is absorbed into the blood stream proportionate to the dose administered and is stored in greater amounts and for longer periods when the dose is massive.[13] Apparently the body does not possess an efficient mechanism for destruction or inactivation of the material when given in excess. Warkany[13] found that, in rabbits, the oral ingestion of 100,000 units of vitamin D increased the normal blood serum value of about 50 units per 100 c.c. of serum to as much as 1,350 units in six hours and 2,700 units in twenty-four hours. If 1,000,000 units of viosterol was administered orally, values as high as 54,000 units per 100 c.c. of blood serum were found. These high values were maintained for about four days, and then slowly diminished for a period of four to six weeks before returning to normal. In adult humans, Warkany[13] found that after abrupt withdrawal of high daily doses of vitamin D, from three to six months elapsed before the concentration of vitamin D in the blood serum fell to a normal level.

Heymann[13] in 1936 found detectable amounts of vitamin D circulating in the blood from two to three months after the administration of a single oral dose of 200,000 units of vitamin D. The following year he showed that if rabbits were given a similar dose and the animals killed in from one to twelve weeks, the vitamin was stored in the tissues. Vitamin D was found in the brain from one to two weeks afterward, in the erythrocytes five to six weeks, in the lungs six to nine weeks, the kidneys six to nine weeks, the liver six to twelve weeks, and blood plasma eight to twelve weeks or more.

There is conflicting evidence as to which tissues store vitamin D, but it seems most probable that the vitamin is stored in the blood serum, skin and fat, lungs and kidneys. The tissues of certain children, dying of tuberculosis or leucemia, to whom massive doses of vitamin D had been given weeks or days before death, have been studied biologically in feeding experiments to rats.

In such a study, Windorfer[14] found that the kidneys and brain stored the vitamin. Vollmer,[15] in similar biologic experiments in one case, found the vitamin to an extent of less than 20 U. S. P. units per gram of fresh tissue in the liver and skin. In another case, the skin contained about 15 units (U. S. P.) and the thyroid and parathyroids less than 15 units. In a third case, biologic assays of the skin

and brain showed no evidence of vitamin at an assumed assay level of 15 units per gram of fresh tissue. He was impressed by the relatively small amounts of vitamin D in the tissues compared with the doses administered. He concluded that the vitamin may undergo chemical change in the organism and may be stored in a different form.

Safety of Single Massive Doses of Vitamin D.—The margin of safety in administering large doses of vitamin D is very great, and no fear need be entertained in using massive doses unless toxic levels are maintained for a period of time. It may be pointed out at once that much of the early literature on the toxicity of vitamin D has to be discarded because impure preparations such as the old vigantol, containing toxisterols, were used, and these early preparations were not standardized as to potency of vitamin D. It is now well known that if ergosterol is overirradiated, toxisterols are produced which are low in antirachitic value and high in toxic properties. The old vigantol, according to the manufacturer's specifications, was required to be irradiated until toxisterol (substance 248) in maximum concentrations was produced.[16] The same question must be considered today in evaluating the reports of vitamin D toxicity. For example, Tumulty[17] recently reported toxic effects in two patients receiving large doses of vitamin D. The product he used, however, contained 50 per cent luminosterol and toxisterols.

Other reports in the literature citing toxic effects of large or even small doses of vitamin D in children have to be evaluated in the light of our present knowledge of the contributing role played by kidney disease. If renal disease, such as chronic interstitial nephritis, polycystic kidneys or hydronephrosis or pyelonephritis, exists in infants or young children, the use of large doses of vitamin D predisposes to metastatic calcification. In such cases, too small doses of vitamin D may result in renal rickets or if larger doses of vitamin D are administered, doses which under ordinary circumstances are perfectly safe, metastatic calcification of the organs and blood vessels may follow.[18] Experimental work in rats has also shown the importance of renal damage on the toxicity of vitamin D.[19]

Calcification of the arteries and organs may occur in infants as the result of disturbances of calcium metabolism due to other factors than vitamin D. Kidney disease is frequently present. Hild[20] recently reported the occurrence of calcification of the arteries and lungs in an infant with renal insufficiency who received subminimal doses of vitamin D. Baggenstoss and Keith[21] reported the case of an 8-week-old female infant who received no vitamin D and yet showed extensive calcification of the arteries, heart and kidneys. He reviewed nine other similar cases described in the literature, in four of whom kidney disease was present.

Much work has been done to determine the level of toxicity of vitamin D in animals with the use of purified preparations. The range between therapeutic efficacy and toxicity is very great. In the rat,

Bills and Wirick[22] in long-time feeding experiments showed that a dosage of 1,000 times that of the minimum rachitic level was just perceptibly harmful; 4,000 times overdosage of viosterol was definitely injurious, and 40,000 times overdosage was strongly toxic. Others,[23] Rosenheim and Webster (1927), Harris and Moor (1928), Light, Miller, and Frey (1929), Collazo, Rubin, and Varel (1928), and Harris, Ross, and Bunker (1939), have placed the toxic level in animals on short-time feeding experiments between 5,000 and 100,000 times overdosage as lethal. Steck, Reed, and Struck, in 1937,[24] reported their observations on sixty-four dogs and 773 human subjects receiving massive doses of vitamin D. They concluded that it was important to record data as to the dose per unit of body weight. Both human beings and dogs generally survive the administration of 20,000 units per kilogram per day of vitamin D for indefinite periods without intoxication. Levels beyond this are toxic.

The use of large doses of purified preparations of vitamin D in infants and children has been conclusively shown to be safe in single doses or in short-time administration, except in the presence of kidney disease. In 1930 Hess, Poncher, Dale and Klein,[25] using 600 and 1000-D viosterol in twelve infants, six of whom were Negroes, showed that doses 21 to 52 times the prophylactic dose in long-time feeding periods (six to eight months) were not toxic for these infants. They also found that infants tolerated 250 times overdosage well for short periods. No clinical symptoms of toxicity developed. Since 1936, when purer preparations of vitamin D became available, doses ranging from 200,000 to 600,000 units for the treatment and prophylaxis of rickets have been used. No toxic manifestations have been reported.[1-10] Zelson[11] even administered 600,000 units of vitamin D orally and parenterally for rickets prophylaxis to forty-six premature infants between 12 and 23 days of age without observing toxic effects.

Examination of the tissues of infants and children, dying of other diseases, who received single massive doses of vitamin D, as high as two or three million units, also have shown the absence of toxic effects. In 1939, Vollmer[15] gave a 4-year-old Negro boy 600,000 units of vitamin D orally, and 1,000,000 units of vitamin D in 1 c.c. of oil intramuscularly, three and one-half days and thirty-six hours, respectively, before he died of miliary tuberculosis. No evidence of calcification of the tissues could be found. In 1941 he gave a 3-year-old child, who eventually died of lymphatic leucemia, a total of 2,260,000 units of crystalline vitamin D_3 (200,000 units per kilogram) diluted in oil and ether and administered intramuscularly, twenty-six, thirteen, and seven days before death. No evidence of tissue calcification was found. Another colored child, aged 19 months, who died of miliary tuberculosis, was given a total of 1,200,000 units of vitamin D (200,000 units per kilogram) in the form of fish liver oil concentrate without a solvent, intra-

muscularly, nine and then three days before death. No evidence of metastatic calcification was found in the blood vessels or organs.

Even though single massive doses of vitamin D as high as 600,000 units may be given to young infants and higher single doses to older children without toxic effects, such as loss of weight, vomiting, anorexia, or metastatic calcification of soft tissues, yet sufficiently large doses of vitamin D if given for longer periods of time may prove toxic. Steck, Deutsch, Reed, and Struck[26] in their experiments on dogs showed that daily doses above 20,000 units per kilogram weight were toxic. Ross and Williams,[27] in 1937, reported a case of a premature twin between 8 and 14 months of age (a group of four cases were reported and no exact details were given) who received a preparation of irradiated ergosterol, containing no toxisterols, in daily doses of from 20,000 to 40,000 U.S.P. units for several months, probably a total dosage of about three million units. The symptoms that followed were anorexia, loss of weight, and vomiting. The infant died suddenly following a bronchopneumonia, and post-mortem examination of the tissues showed extensive metastatic calcification of the arteries, cardiac muscle, kidney tubules, stomach, and lungs.

From the data cited, it is apparent that for young infants a total massive dosage of about three million units of vitamin D concentrated over a period of time may result in metastatic calcification of the tissues. Older infants and children will tolerate larger doses. However, single large doses, even repeated at occasional intervals, are perfectly safe.

Reed, Struck, and Steck[24] have shown experimentally in dogs that even if calcification is produced in the kidneys, the process is reversible if the administration of vitamin D is stopped. There is also some evidence to show that irradiated cholesterol is less toxic in large doses than irradiated ergosterol. Electrically activated ergosterol (ertron) is less toxic than irradiated ergosterol and may be a different form of vitamin D than calciferol.[24]

Oral Versus Parenteral Administration of Vitamin D.—Vollmer[28] and Zelson[11] have indicated that single massive doses of vitamin D should be administered parenterally rather than orally in order to obviate poor absorption or improper administration on the part of the attendant. It is true that if an oily preparation of vitamin D is incorporated in the formula, it may be found floating on top, or part of the vitamin is discarded if the bottle is not finished. This is not the case if electrically activated ergosterol is used in powder form. This preparation mixes well with cereal and milk and may be administered in a small amount of the food. The milky vehicle aids absorption. It is well taken, causes no digestive upsets, and affords excellent protection against rickets. In the occasional case where the feeding is regurgitated, the dose can be repeated one week later. If the single massive dose is given at the third month, the infant has usually recovered from earlier digestive

upsets. A period is chosen in which the baby is eating well and not vomiting. Under these circumstances there seems to be no need for adding to the already increasing number of injections for infants. If an infant is suffering from a severe intestinal disturbance, or he is a premature whose intestinal absorption is poor, parenteral administration is indicated.

Stimulation of Metabolism.—Rachitic infants who are treated by the single massive dose method show an immediate improvement in their disposition and general condition. Hess, Poncher, Dale, and Klein[25] observed that the feeding of large daily doses of vitamin D for rickets prophylaxis markedly increased the appetite and that weight gains were good, although not in proportion to the food intake. Reed, Struck, and Steck[24] stated in their book on vitamin D that "Vitamin D is intimately related to thyroid function in that deficiency decreases the metabolic rate, and in normal subjects the vitamin is capable of elevating the metabolic rate. This effect is not mediated through the parathyroids but probably through the thyrotropic mechanism of the anterior pituitary. Only in fasting animals can an increased nitrogen output be conclusively demonstrated."

In this present series the observations of others showing the effect of a large dose of vitamin D on the metabolism were confirmed. The appetite was often stimulated and many of the infants made large gains following the administration of the single massive dose. The incidence of respiratory infections was not influenced.

Recommended Dosage for Single Massive Dose Vitamin D Prophylaxis.—The size of the single massive dose of vitamin D necessary for rickets prophylaxis depends upon three factors: (1) age of the infant at the time of administration; (2) weight of the infant; and (3) time interval between doses. In this series of cases the initial single massive dose of vitamin D was administered about the third month of life or later. The weight of these infants varied between ten and sixteen pounds. Six hundred thousand units of vitamin D in the form of electrically activated ergosterol (ertron) was the routine dose. This was well tolerated and caused no ill effects. Rather, the infants fared exceptionally well, and good gains were observed. Zelson[11] administered 600,000 units of vitamin D to premature infants between 12 and 33 days old, whose birth weights ranged between 2 pounds, 11 ounces, and 5 pounds, without ill effect. It seems more advisable to me, however, to give such small infants, or even infants of 1 or 2 months of age, smaller single doses ranging between 50,000 and 300,000 units. In such cases it would be necessary to repeat the massive dose at a later date.

The work of Harnapp, Windorfer, Heisler, and others reviewed in the first part of this paper showed conclusively that infants ranging between the ages of 2 months and 1½ years can be adequately protected against rickets for the duration of the winter on 400,000 units of vitamin D

without subsequent dosage. Mild rickets may develop in a small percentage of cases on a single massive dose of 200,000 or 300,000 units given only once during the winter. Premature infants require at least 400,000 units in a single massive dose. If smaller single doses are used, such as 200,000 units, a second dose during the winter months is required for adequate protection.

The following two plans are suggested for massive dose rickets prophylaxis:

1. The infant can be protected until the second or third month by daily prophylactic doses of 1,000 units of vitamin D. After this, a single dose of 600,000 units of vitamin D should be administered, the latter dose to be repeated four or five months later, depending on the clinical findings, the time of year, and the age of the infant. In the summer an older infant may be allowed to go six months before repeating the massive dose.

2. At the end of the first and second months of life, respectively, 50,000 units of vitamin D may be administered in milk. About the third month, after pre-cooked cereals are started, 600,000 units may then be administered in the cereal and milk, the massive dose to be repeated four or five months later, depending on the physical or roentgen findings, age, and time of year. Older children can be given 600,000 units of vitamin D twice a year.

Either of these plans of prophylaxis eliminates the administration of excessive doses before the second month. In any event, if minimal rickets has developed on daily doses of vitamin D, the administration of a single massive dose at the third month heals the process quickly and gives further protection for about five months.

SUMMARY

Although single massive doses of vitamin D were used experimentally as early as 1928 for the prevention of rickets in experimental animals, it was not until 1938 that this method of rickets prophylaxis was applied to infants. Since that time numerous workers in Germany and elsewhere have utilized this procedure with effective results. When at least 400,000 to 600,000 U.S.P. units of vitamin D were administered orally or parenterally to normal or premature infants even as young as one month, effective protection against rickets for the duration of about five winter months occurred. These workers stressed the safety of this method.

In this series, seventy-five infants were given 600,000 U.S.P. units of an electrically activated preparation of ergosterol (ertron), divided in two oral doses in precooked cereal and milk at about the third to the fifth month of life. Only sixty-two of these infants could be followed throughout the entire period. At the onset of the study, eighteen showed mild rickets which healed during the period of observation of from two to seven months after treatment and remained healed. Forty-

four other infants were followed for from two to seven months after the single massive dose of vitamin D and showed no evidence of rickets during this observation period.

Massive doses of vitamin D administered in the form of electrically activated ergosterol (ertron) were well tolerated, and no toxic manifestations were observed, such as loss of weight, vomiting, or anorexia. Rather, these infants thrived exceptionally well. No diminution in the incidence of respiratory infections was noted. There is no danger in administering single massive doses of vitamin D to young infants, even premature infants. The danger lies in repeating these large doses over a period of time so that several million units of vitamin D are given. Depending on the age and weight of the infant, such large doses may be toxic if repeated daily.

Two plans for administering single massive vitamin D prophylaxis against rickets are outlined. One is to protect the infant until 2 or 3 months of age by means of daily prophylactic doses of 1,000 units of vitamin D and then to administer a single dose of 600,000 units of vitamin D. The latter dose may be repeated from four to six months later. The other plan is to give an infant at 1 and 2 months of age, respectively, 50,000 units of vitamin D in formula, and then at about 3 months of age to administer a single dose of 600,000 units in precooked cereal, which single dose may be repeated from four to six months later.

There is experimental evidence to show that vitamin D when given in single massive doses is stored in the blood plasma and the tissues for a period of weeks or months. This experimental evidence is borne out by the clinical observations of this series of cases and others. The oral single massive dose prophylaxis of rickets is to be preferred to parenteral injection for the average case. Only in cases of severe intestinal disturbances or in the case of young premature infants whose absorption may be poor does it seem necessary to use the parenteral method.

The administration of a single massive dose of vitamin D for the prophylaxis of rickets is not only of proved effectiveness, but also is safe and time-saving.

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SUSCEPTIBILITY OF SHIGELLA PARADYSENTERIAE TO SODIUM SULFATHIAZOLE AND SULFAGUANIDINE IN VITRO

WITH SOME INFORMATION REGARDING EBERTHELLA TYPHOSA, SALMONELLA PARATYPHOSA, ESCHERICHIA COLI, SALMONELLA MORGANI AND PROTEUS

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THE purpose of this study was to compare the bacteriostatic and bactericidal concentrations in vitro of sodium sulfathiazole* and sulfaguanidine* for cultures of *Shigella paradysenteriae* Flexner, *Shigella paradysenteriae* Sonne, *Shigella alkalescens, Escherichia coli, Eberthella typhosa, Salmonella paratyphi, S. morgani,* and Proteus. All of these cultures had been isolated from the stools of children acutely ill.

This study differs from other in vitro studies in two respects: first, the observations were made over a period of twenty-one to twenty-eight days instead of one, two, or three days as is the case in most reports; and second, each culture was studied in a series of at least nineteen different concentrations of the drugs while most reports are based on one, two, or three concentrations of the drugs. Lawrence[1] used only one short period of observation, twenty-three to twenty-five hours, and only one concentration of sulfonamide, 10 mg. per cent. His data indicated that sulfathiazole and sulfamethylthiazole were somewhat more bacteriostatic over this short incubation period than were sulfapyridine and sulfanilamide on cultures of *E. typhosa, Esch. coli, S. dysenteriae, S. paratyphi, S. suipestifer, S. enteritidis, A. aerogenes,* and *Proteus vulgaris.* Remmelkamp and Jewell[2] made their observations at the end of a twenty-four-hour incubation period and used only one concentra- of sulfonamide, 10 mg. per cent. They used cultures of *E. typhosa S. paratyphi,* and *Shigella paradysenteriae* Flexner and Sonne in broth medium containing sulfathiazole, sulfanilamide, sulfapyridine, and sulfamethylthiazole. Their results showed that there was some bacteriostatic effect on *S. paratyphi* and *Shigella paradysenteriae* Flexner when the inoculum was about ten microorganisms per cubic centimeter. Bornstein and Straus[3] observed at the end of twenty-four hours that sulfanilylguanidine was bacteriostatic in less than 10 mg. per cent for *Shigella paradysenteriae* Flexner and *S. cholerae suis,* in 10 mg. per cent concentration for *S. paratyphi A,* and in 25 mg. per cent for *E. coli.* Long and Bliss[4] made their observations at the end of from seven-

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*Kindly supplied by E. R. Squibb and Sons.

teen to twenty-four hours on the bacteriostatic effect of 10 mg. per cent of sulfathiazole, sulfapyridine, and sulfanilamide on cultures of *Esch. coli* and *B. proteus*. They found sulfathiazole as effective as sulfapyridine. Lawrence and Sprague[5] used concentrations of sulfanilamide, sulfapyridine, sulfathiazole, and sulfanilylguanidine varying from 100 to 200 mg. per cent. They observed that sulfathiazole was more effective in vitro as to bacteriostatic and bactericidal activity upon bacteria of the colon-typhoid-dysentery group. Straus and Finland[6] obtained viable bacterial counts after six, twelve, and twenty-four hours and observed that with small inocula sulfadiazine and sulfathiazole were bactericidal in concentrations as low as 1 or 2 mg. per cent on *Esch. coli* and *Shigella* paradysenteriae Flexner, and in 5 mg. per cent on *S. schott*-mülleri. Both drugs were bacteriostatic in 5 mg. per cent for *S. paratyphi*.

Bacto nutrient broth plus sulfonamide was the medium used in this study, and the inoculum was usually between 50 and 150 microorganisms per cubic centimeter. Our cultures are stored in a semisolid medium composed of bacto brain heart infusion plus 0.3 per cent nutrient agar, in which heavy growth occurs. Two serial subcultures were made from the semisolid culture into synthetic medium which afforded moderate growth. The second culture was diluted 1:100,000 with nutrient broth, and 0.2 c.c. of this was inoculated into each of a series of tubes containing 10 c.c. of nutrient broth with various concentrations of sulfonamide. The different concentrations of sulfonamide were obtained by first preparing the highest concentration in nutrient broth. This was then sterilized in the autoclave and all other concentrations were prepared by making dilutions in sterile nutrient broth. The inoculated tubes of sulfonamide broth were incubated at 37° C. for at least three weeks, observed frequently, and subcultures made at regular intervals to determine the bactericidal concentration.

Daily observations of broth cultures containing increasing concentrations of sulfonamide show increasing bacteriostatic levels. If such observations are made over a sufficient number of days, the final bacteriostatic concentration is the same as the final bactericidal concentration. Bacteriostatic and bactericidal sulfonamide in vitro data are influenced greatly by certain factors which we have pointed out recently[7] such as the number of bacteria in the inoculum, the quality of the medium and the length of the period of observation. Any period of observation less than that necessary to give data regarding the final bacteriostatic and final bactericidal concentrations provides data which is incomplete and dependent on the time of observation. We feel that our final results justify carrying the experiment through to completion. It is our experience that the comparison of such final data for a number of strains of bacteria is significant, and we have found that fairly constant results may be obtained by repetition under similar controlled conditions. A similar method could be developed which would be ap-

TABLE I

SHIGELLA PARADYSENTERIAE FLEXNER

BACTERIOSTATIC AND BACTERICIDAL CONCENTRATIONS OF SODIUM SULFATHIAZOLE AND SULFAGUANIDINE IN VITRO

STRAINS	INOCULUM BACTERIA PER C.C.	SODIUM SULFATHIAZOLE BACTERIOSTATIC CONCENTRATIONS (MG. PER CENT)						SULFAGUANIDINE BACTERIOSTATIC CONCENTRATIONS (MG. PER CENT)					FINAL BACTERICIDAL CONCENTRATIONS (21 DAYS)	
		DAYS						DAYS					SODIUM SULFATHIAZOLE	SULFAGUANIDINE
		1	2	4	7	14	21	1	2	4	7	14		
B "R" 88	51	<1	<1	<1	<1	30	30	5	20	30	50	50	80	130
B R	46	0.1	0.1	1	1	30	50	5	20	20	150	>200*	100	>200*
J. K. I	88	0.1	0.1	1	20	40	40	5	5	>200*	>200*	>200*	40	>200*
J. K. 5 88	85	0.1	0.1	1	40	50	70	5	5	170	170	>200*	70	>200*
D. B. I	78	0.1	0.1	1	50	50	50	5	80	90	200	200	50	200
D. B. 5 88	96	0.1	0.1	1	1	10	10	5	80	80	>200*		10	>200*
V. O. I	140	0.1	0.1	0.1	10	20	20	5	10	70	100	200	20	>200*
V. O. 5 88	150	0.1	1	1	10	30	30	5	10	100	>200*		30	>200*
P. W. I	110	0.1	0.1	1	50	60	70	5	5	10	170	200	120	>200*
P. W. 23	106	0.1	0.1	1	70	90	140	5	5	30	170	200	140	>200*
P. S. 21	119	<0.1	<0.1	<0.1	50	200	200	5	5	10	>200*		200	>200*
P. S. 10	67	1	20	40	120	160	160	30	200	>200*	>200*		160	>200*

*Higher concentrations necessary for bacteriostatic and bactericidal effects.

plicable as a standard technique for testing the final bactericidal and changing bacteriostatic concentrations of sulfonamides for various bacteria. As conditions are now, each investigator in this field uses his own choice of media, number of bacteria inoculated, quantity of drug, and time of observation, and he usually observes only for bacteriotasis. More recently we have determined by bacterial plate counts that gross evidence of growth in our sulfonamide broth media does not become apparent until the bacterial count reaches 15 million *Shigella paradysenteriae* per cubic centimeter. Thus it would seem that evidence of growth in sulfonamide broth media is not a delicate measurement of the bacteriostatic influence of a drug on a microorganism. Profuse multiplication must occur before bacteriostasis is ruled out by this method.

In Table I is seen data regarding the changing bacteriostatic and the final bactericidal concentrations of sodium sulfathiazole and sulfaguanidine for the twelve Flexner strains. The bacteriostatic effect was pronounced until the seventh day for all strains except one. During subsequent days growth appeared in the tubes of broth containing increasing concentrations of the sulfonamide approaching the bactericidal concentration. The Flexner strains showed a wide variation in susceptibility to sodium sulfathiazole. Bactericidal concentrations as low as 10 mg. per cent and as high as 200 mg. per cent were observed for different strains. Eight of the twelve strains were killed by 100 mg. per cent or less of the sulfonamide in medium. The other four strains were killed by the sulfonamide in concentrations between 100 and 200 mg. per cent. All 12 strains of *Shigella paradysenteriae* Flexner were killed by concentrations of sodium sulfathiazole varying between 10 and 200 mg. per cent. Such results are in keeping with clinical therapeutic results published previously[8] in which it was shown that children with bacillary dysentery derived benefit from the use of sulfathiazole and those with nonspecific diarrhea did not derive benefit from its use. All of the Flexner strains showed growth earlier and more rapidly in medium containing sulfaguanidine than in similar medium containing sodium sulfathiazole. Only two of the twelve strains were killed by 200 mg. per cent of sulfaguanidine in medium. The solubility of sulfaguanidine is 200 mg. per cent.

The seven strains of *Shigella paradysenteriae* Sonne, Table II, were much less susceptible to both sulfonamides than were the Flexner strains. The Sonne strains showed growth earlier in medium containing higher concentrations of both sulfonamides than did the Flexner strains. It was necessary to repeat studies on three strains with higher concentrations of sodium sulfathiazole in the medium in order to obtain an end point for the bacteriostatic and bactericidal concentrations, since in the first studies with these particular strains growth occurred in medium containing all concentrations of the sulfonamide. The two sets of data for each of

TABLE II

SHIGELLA PARADYSENTERIAE SONNE BACTERIOSTATIC AND BACTERICIDAL CONCENTRATIONS OF SODIUM SULFATHIAZOLE AND SULFAGUANIDINE IN VITRO

STRAINS	INOCULUM BACTERIA PER C.C.	SODIUM SULFATHIAZOLE BACTERIOSTATIC CONCENTRATIONS (MG. PER CENT) DAYS						SULFAGUANIDINE BACTERIOSTATIC CONCENTRATIONS (MG. PER CENT) DAYS				FINAL BACTERICIDAL CONCENTRATIONS (21 DAYS)	
		1	2	4	7	14	21	1	2	4	7	SODIUM SULFATHIAZOLE	SULFAGUANIDINE
G. B. I	86	1	30	120	170	>200*	>200*	50	>200*	>200*	>200*	>200*	>200*
G. B. I	89	5	5	30	120	270	350					350	
G. B. 3 88	112	1	40	90	170	>200*	>200*	90	>200*	>200*	>200*	>200*	>200*
G. B. 3 88	100	5	30	60	300	300	350					350	
R. W. I	82	0.1	30	60	120	250	300	20	>200*	>200*	>200*	400	>200*
R. W. 18 M	78	0.1	50	90	170	350	400	20	>200*	>200*	>200*	400	>200*
Ch.	36	2	10	60	>200*	>200*	>200*	80	>200*	>200*	>200*	>200*	>200*
Ch.	105	1	30	60	350	350	350					400	
P. S. I	97	5	5	70	250	400	400	160	>200*	>200*	>200*	400	>200*
Ma. I	60	1	5	40	80	160	160	30	160	200	>200*	250	200

*Higher concentrations necessary for bacteriostatic and bactericidal effects.

TABLE III

EBERTHELLA TYPHOSA, SALMONELLA PARATYPHI, SALMONELLA MORGANI, SHIGELLA ALKALESCENS, ESCHERICHIA COLI, AND PROTEUS
BACTERIOSTATIC AND BACTERICIDAL CONCENTRATIONS OF SODIUM SULFATHIAZOLE AND SULFAGUANIDINE IN VITRO

STRAINS	INOCULUM BACTERIA PER C.C.	SODIUM SULFATHIAZOLE BACTERIOSTATIC CONCENTRATIONS (MG. PER CENT)						SULFAGUANIDINE BACTERIOSTATIC CONCENTRATIONS (MG. PER CENT)					FINAL BACTERICIDAL CONCENTRATIONS (21 DAYS)	
		DAYS						DAYS					SODIUM SULFATHIAZOLE	SULFAGUANIDINE
		1	2	4	7	14	21	1	2	4	7	14		
Esch. coli B)	153	100	>400*	>400*	>400*	>400*	>400*	200	>200*	>200*	>200*	>200*	>400*	>200*
Esch. coli B(	40	100	>2000*										>2000*	
Esch. coli M	74	70	140	180	250	300	350	100	>200*	>200*	>200*	>200*	350	>200*
Esch. coli N.	130	1	5	60	180	350	350	40	100	>200*	>200*	>200*	350	>200*
Esch. typhosa P.	60	1	5	450	550	600	600	40	140	>200*	>200*	>200*	600	>200*
Esch. typhosa O	90	1	5	180	250	300	300	40	180	>200*	>200*	>200*	300	>200*
S. paratyphi B. L-1	105	20	250	400	450	550	550	100	>200*	>200*	>200*	>200*	600	>200*
S. 10-88-1	76	20	30	80	120	200	200	30	>200*	200*	>200*	>200*	250	>200*
S. paratyphi A	87	1	5	5	250	250	350	30	50	90	180	>200*	350	>200*
S. paratyphi B)	68	5	250	600	800	800	>800*	70	>200*	>200*	>200*	>200*	>800*	>200*
S. paratyphi B(	80	1	250	750	850	850	900						900	
S. morgani B)	162	20	20	150	300	>400*	>400*	180	>200*	>200*	>200*	>200*	>400*	>200*
S. morgani B(	100	5	30	250	400	500	500						500	
S. morgani N	290	1	5	5	40	60	60	60	90	>200*	>200*	>200*	60	>200*
Shigella alkalescens Br.	82	1	40	80	120	120	120	100	180	>200*	>200*	>200*	120	>200*
Shigella alkalescens Be.	87	1	10	90	100	100	120	40	90	>200*	>200*	>200*	120	>200*
Proteus J.	118	0.1	0.1	5	40	70	70	5	10	>200*	>200*	>200*	70	>200*
Proteus N.	276	0.1	1	10	20	70	70	5	10	180	>200*	>200*	70	>200*

*Higher concentrations necessary for bacteriostatic and bactericidal effects.

these three strains are included in Table II to show that these data are characteristic of strains of the particular bacteria and not merely culture differences of the same strain. We have had occasion to repeat our sulfonamide studies on other strains, and the results obtained on repetition have always been similar to those obtained previously. The bactericidal concentrations for the Sonne strains varied between 250 and 400 mg. per cent in contrast to the Flexner strains, where all but one were killed by concentrations of the drug less than 200 mg. per cent. Sulfaguanidine in the medium had only a slight and brief bacteriostatic effect on the Sonne cultures. All but one of the seven Sonne strains tested in sulfaguanidine showed growth on the second day in medium containing 200 mg. per cent of the drug, the maximum concentration used. This one strain showed growth in medium containing 200 mg. per cent of the drug on the fourth day. None of these strains were killed by 200 mg. per cent of sulfaguanidine in medium.

The miscellaneous group of fifteen strains, Table III, composed of *Esch. coli*, *E. typhosa*, *S. paratyphi*, *S. morgani*, *Shigella alkalescens*, and Proteus varied in their susceptibility to the bacteriostatic effect of sodium sulfathiazole. Twelve of these fifteen strains showed heavy growth within seven days in medium containing high concentrations of this drug. Eleven of these twelve strains were killed by concentrations of sodium sulfathiazole varying between 120 and 900 mg. per cent in medium. One strain, *Esch. coli*, was not killed by 2,000 mg. per cent. The other three strains, one of Proteus and two of *S. morgani*, were quite susceptible to the bacteriostatic effect of sodium sulfathiazole and were killed by 60 and 70 mg. per cent of the drug in medium. Again sulfaguanidine in the medium had only slight and brief bacteriostatic effect on all these cultures. All but two strains showed heavy growth within four days in 200 mg. per cent of sulfaguanidine in broth. Sulfaguanidine in 200 mg. per cent concentration was not bactericidal for any of the cultures in this group. Our strains of *E. typhosa* and *S. paratyphi* had been isolated from children acutely ill with these infections who were subsequently treated with sulfathiazole or sulfaguanidine or both separately without any favorable response. One strain of *Shigella alkalescence* (Br.) had been isolated from a patient with recurring severe bloody diarrhea. This patient was treated with large doses of sulfaguanidine over a number of days without any improvement clinically and without the disappearance of the microorganism from the stool.

SUMMARY

Studies are reported on the bacteriostatic and bactericidal effects of sodium sulfathiazole and sulfaguanidine in vitro on twelve strains of *Shigella paradysenteriae* Flexner, seven strains of *Shigella paradysenteriae* Sonne, and fifteen strains of a miscellaneous group of intestinal bacteria, most of them pathogenic.

These studies differ from preceding studies in that the observations were made over a period of twenty-one days rather than one or two days, as is the usual custom, and each strain was studied in a series of at least nineteen different concentrations of the drugs instead of one, two, or three concentrations as is usual.

Sulfaguanidine was bactericidal for only two of the thirty-four different strains tested, and these were both strains of *Shigella paradysenteriae* Flexner.

Sodium sulfathiazole was bactericidal for all strains tested except one strain of *Esch. coli* which survived in media containing 2,000 mg. per cent of the drug.

For the Flexner strains, sodium sulfathiazole was bactericidal for all in concentrations between 10 and 200 mg. per cent inclusive.

For the Sonne strains, sodium sulfathiazole was bactericidal for all in concentrations between 250 and 400 mg. per cent inclusive.

For the miscellaneous group, sodium sulfathiazole was least bactericidal for strains of *Esch. coli*, *E. typhosa*, *S. paratyphi*, and two of the *S. morgani*. The bactericidal concentrations for these varied between 250 and more than 2,000 mg. per cent. For one strain of *S. morgani*, two of *Shigella alkalescens*, and two of Proteus, the bactericidal levels were between 60 and 120 mg. per cent, which are comparable to those observed for the Flexner cultures.

CONCLUSION

These results indicate that sodium sulfathiazole was more bacteriostatic and bactericidal for Flexner strains of *Shigella paradysenteriae* than for Sonne strains and still less bactericidal for *E. typhosa* and *S. paratyphi*.

These results also indicate that sodium sulfathiazole was much more bactericidal than sulfaguanidine for the strains tested.

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HISTAMINASE IN THE TREATMENT OF ALLERGY IN CHILDREN

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THE literature on histaminase in allergy deals largely with adults. The therapeutic efficacy of histaminase is in a state of flux. In view of the numerous inquiries we continue to receive from pediatrists and general practitioners concerning the value of histaminase in the treatment of allergic disorders of childhood and because of the well-known tendency of children to respond favorably and readily to specific and nonspecific measures used for the control of allergic disorders, we felt that our experiences with histaminase in allergic children were worthy of report.

In 1930 Best and McHenry[1, 2] isolated an enzymelike substance which, when incubated with histamine, caused the latter to become neutralized. This substance they termed histaminase. It was isolated as a dry powder from the kidney and the intestinal mucous membrane. In 1936 an extract with the properties said to be similar to those of the histaminase of Best and McHenry was introduced into this country from Germany for clinical trial in allergic conditions. At this time, Brown, Roth, and Horton[3] reported favorable results of treatment with histaminase in patients with physical allergy. They held that the causative agent in this condition was histamine, a histamine-yielding substance, or a histamine-like substance.

In 1938, histaminase was prepared and distributed by its manufacturer in this country. Since then, histaminase has been employed liberally for the treatment of all types of allergic disorders. Many reports soon appeared in the literature on the success obtained following the oral administration of histaminase for such conditions as serum sickness,[4, 5] allergic dermatosis,[6, 7, 8] vasomotor rhinitis,[9, 10, 11] hay fever,[10] asthma,[12] physical allergy,[13, 14, 15] and erythromelalgia of the head (histamine cephalgia).[16] However, angioneurotic edema and urticaria[17] and physical allergy[3, 15] were the conditions reported giving the best response. In contrast to the foregoing favorable reports, there is a rapidly accumulating literature indicating uniform failure of histaminase for the various allergic conditions treated. Many of these reports showed that their authors made a diligent effort to determine dosage and em-

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The histaminase used in this study was supplied as Torontil by the Winthrop Chemical Co., New York.

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ployed careful judgment in the evaluation of the results. Miller and Piness[18] treated forty-two allergic patients (twenty-eight with urticaria). Keeney[19] reported on fifteen patients with hay fever due to ragweed. Vaisberg[20] reported failures in patients with asthma, migraine, urticaria, and vasomotor rhinitis but points out that histaminase therapy may prove of value in serum sickness, physical allergy, and idiopathic pruritis.

MATERIALS AND METHODS

This study is comprised of forty-eight allergic children (thirty-two boys and sixteen girls) between 3 and 16 years of age.

A daily dose of 50 units of histaminase in enterically coated tablets was administered to all patients. Each tablet contained 5 units of the dry powder derived from the mucosa of the small intestines and kidney of the hog. Ten tablets were divided into three doses and given before meals. Treatment was maintained for periods of from two to twenty weeks or an average of nine weeks per patient.

The majority of the children prior to the administration of histaminase had been under treatment for two or more years according to the accepted standard of allergic practice. All of these children maintained dietary and environmental restrictions during the period of treatment with histaminase, while a number of them were also given pollen and other antigens subcutaneously.

The children were observed about once a week. Careful clinical notes were made and the results of treatment recorded by each worker.

REACTIONS

No patients in this series had a positive skin reaction to pork or a history of clinical intolerance to hog meat. However, two patients not included in this study discontinued the use of histaminase because it caused nausea, vomiting, and abdominal colic. These children showed negative cutaneous reactions as well as a negative history of intolerance to pork.

RESULTS

All of the forty-eight children comprising this study failed to be relieved from the allergic syndrome for which histaminase therapy was given despite the fact that adjuvant dietetic and environmental adjustment and other specific measures were employed. Moreover, those patients with more than one allergic syndrome even failed to show relief from one of their syndromes.

Twenty of the forty-eight children in this study had two or more allergic syndromes, the total number being 80, as follows: Asthma (nonpollen), 28; hay fever, 26; vasomotor rhinitis, 19; urticaria, 3; eczema, 2; migraine, I; and physical allergy to cold (angioneurotic edema), 1. However, histaminase was administered for the treatment of the allergic syndromes which did not respond to specific therapy or

for which specific therapy was not used. The number of allergic syndromes treated with histaminase totaled sixty-three and were as follows: asthma, 26; vasomotor rhinitis, 18; hay fever, 12; urticaria, 3; eczema, 2; migraine, 1; and physical allergy to cold (angioneurotic edema), 1. The pertinent facts in the treatment of these syndromes were as follows:

Perennial asthma was present alone in 12 children. The asthma was aggravated during the cold months of the year when treatment with histaminase was commenced. The period of treatment averaged three months per patient.

Perennial asthma and ragweed hay fever occurred in three patients. In two of these children histaminase was given over a period of two months in the fall of the year for the treatment of the asthma. In one of these two children ragweed pollen antigen failed to relieved the symptoms. In the remaining child, ragweed desensitization was not done, but histaminase was started three weeks prior to the pollinating season and maintained for four months.

Perennial asthma, hay fever, and vasomotor rhinitis occurred in nine children. In six of these children, treatment with ragweed antigen afforded relief from hay fever. However, histaminase was given for the control of asthma and vasomotor rhinitis in the fall of the year for an average period of forty-two days per patient. In another two patients histaminase was given for one month before the ragweed season. In one of these two children the enzyme was continued through the pollinating season and for seven weeks thereafter. In the remaining case, histaminase was administered only for two weeks during the pollinating season.

Eight children had ragweed hay fever. None of them were given specific pollen treatment. Five of these children received histaminase for a total average period of two months per child, treatment commencing for an average period of twenty-one days before the pollinating season. In the remaining three patients the enzyme was started during the pollinating season (and continued for some time thereafter) for a total average period of fifty-one days per patient.

Vasomotor rhinitis and hay fever occurred in four children who received specific pollen antigen. Relief from hay fever was obtained in three of these patients who were given histaminase for the control of the vasomotor rhinitis. In these three children the enzyme was administered after the pollinating season and continued for an average period of fifty-five days per patient. In the fourth child, histaminase was started three weeks before the pollinating season and continued to the late fall for a total period of four and one-half months.

Vasomotor rhinitis alone was present in four patients. Treatment with histaminase was commenced in October and given for an average period of fifty days per patient.

One child was suffering with perennial asthma, hay fever, and chronic eczema. The hay fever was controlled with specific therapy. The asthma and eczema were treated with histaminase which was started in October and given for a period of three months. Asthma and chronic eczema occurred in another child who received the enzyme for thirty-nine days.

Asthma and vasomotor rhinitis occurred in one patient in whom histaminase was started in the summer and given for five months.

Asthma and urticaria occurred in one child who received histaminase for seventy-five days. Another child had vasomotor rhinitis, hay fever (grass and ragweed), and urticaria, in whom pollen antigen controlled the hay fever. Histaminase was administered in the late fall over a period of fifty-six days. Urticaria alone occurred in one child in whom the enzyme was given for two months during the summer.

One patient had migraine for which histaminase was given for one month.

The last child of the series had physical allergy to cold (angioneurotic edema). She has been under treatment for a number of years to no avail when, in December, histaminase was started and continued for forty-four days.

DISCUSSION

The basis of the use of histaminase for the treatment of allergic disorders depends upon the assumption that histamine or H-substance is a factor in the causation of lesions—smooth muscle spasm and localized edema—characteristic of anaphylaxis and allergy. Many practitioners have accepted the above statement as an established fact and so have freely used and continue to use histaminase for the control of allergic conditions despite the fact that it has not been established that histamine is the causative agent in any pathologic condition. Best and McHenry,[1] in 1930, remarked that even if it should be established that the ability of an organism to inactivate histamine can be increased by the administration of the enzyme, there still would be no need for its clinical application. In 1940 Best and McHenry[21] emphasized that they have at no time suggested that the administration of histaminase might be a useful therapeutic measure. Investigations by these observers have failed to show that the administration of histaminase by any route had any effect on the measurable amount of histamine in the body and concluded that there was no physiologic basis on which to rest the use of histaminase in clinical practice. Best and McHenry,[21] Alexander and Bottom,[22] Knoll,[23] Younger, Freeman, and Nungester,[24] Hawes, Alles, and Miller,[25] among others, have been unable to confirm the results of Karody and Browne[26] in the protection of guinea pigs against anaphylactic and histaminic shock with histaminase. There is no conclusive evidence to prove that an excess of histamine is invariably associated with various allergic conditions. Besides, there has been difficulty in determining

the mechanism by which the organism may dispose of an excess of histamine. The work of Rose and Browne[27] is interesting since it suggests that factors other than histaminase may defend the organism against the action of histamine. Recently, Katz[28] has demonstrated that blood cells of sensitized animals released part of their histamine content into the blood plasma when mixed in vitro with the related antigen. In an investigation[29] in allergic individuals with circulating skin-sensitizing antibodies (reagins), an increase in the plasma histamine was observed following incubation of the whole blood with the specifically related antigen. There was also some evidence to indicate that histamine is not the only active substance which is released during this antigen-antibody reaction.

A careful survey of the literature forces the conclusion that histamine has not been definitely established as the exciting cause of any disease. Moreover, the theory that histamine is the common denominator through which a variety of exciting causes can produce certain allergic states has likewise not been established.

The decreased potency of the early batches of histaminase dispensed by the manufacturer was pointed to as the cause of the varying results reported in the literature. However, the histaminase employed in our study has been used with the assurance by its manufacturer that the product was potent and stable. We considered that 50 units of histaminase given daily over an average period of nine weeks per patient was a sufficient period to determine the efficacy of this substance, notwithstanding the fact that the average period for administration of histamine to produce effective results as reported in the literature was much less. From a standpoint of therapeutic usefulness, histaminase did not even measure up to the various nonspecific substances employed by us because not one of the forty-eight children in this study showed a favorable response to histaminase. Thus, the failure of the oral administration of histaminase as a therapeutic agent for the relief of allergic disorders of childhood lends support to the original statement of Best and McHenry who have maintained that there was no place for this enzyme in clinical practice.

CONCLUSIONS

Forty-eight allergic children (thirty boys and eighteen girls) between the ages of 3 and 16 years, suffering with various allergic disorders, were given orally 50 units of histaminase daily for from two to twenty weeks or an average of nine weeks per patient.

Histaminase proved ineffective as a prophylactic, phylactic, or adjunctive therapeutic agent in all the children treated.

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FATAL AGRANULOCYTOSIS FOLLOWING SULFATHIAZOLE THERAPY

Report of Infant with Toxic Dermatosis

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AGRANULOCYTOSIS, a serious and usually fatal complication of sulfonamide intoxication developing in the course of specific chemotherapy, has been observed in from 0.1 and 0.3 per cent of patients treated with sulfanilamide as well as sulfapyridine; its occurrence in those under sulfathiazole or sulfadiazine therapy had not been reported prior to the publication of a tabulation by Long and Bliss[1] and a review by Spink and Hansen.[2] However, during the past year several cases of extreme and fatal granulocytopenia following the therapeutic use of sulfathiazole have appeared in the literature. Only one patient reported by Pippin[3] (Case 3), who received daily doses of sulfathiazole varying from 6.0 to 9.0 Gm. for fifteen days for treatment of pneumonia with pleural effusions and developed acute agranulocytosis, recovered completely after the cessation of medication. Two other patients with fatal agranulocytosis following sulfathiazole therapy, one reported by Kennedy and Finland[4] and the other by Hoyne and Larimore,[5] were given estimated total doses of 120 and 100 Gm. of the sulfonamide over periods of from twenty days to two months, respectively. Both of these patients were adult subjects, the former being a 28-year-old female and the latter a 34-year-old male. As far as we are aware, no cases of acute agranulocytosis in infants and children resulting from sulfathiazole intoxication or sensitization have as yet been recorded. The case here described is the first such instance to be reported occurring in an 8-week-old infant; the clinical manifestations as well as necropsy findings of this patient reveal certain features of unusual interest, not only to the pediatrician but to the general practitioner who employs sulfathiazole for specific chemotherapy.

REPORT OF CASE

The patient, R. H., an 8-week-old male infant, was brought to the University of Chicago Clinics Oct. 29, 1941, because of a nasal condition characterized by the formation of large hard crusts in both nostrils. The baby, the second of twins, had been born normally on Sept. 9, 1941, with no postnatal complications. An attack of diarrhea developing at the age of 3 weeks was satisfactorily treated by feedings of protein milk; but a few days later the infant contracted both impetigo and thrush from his twin sister. Although these conditions improved under proper

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Dr. Elfriede Horst of Des Plaines, Illinois, who first saw the patient, and Dr. C. Anderson Aldrich of the Children's Memorial Hospital, Chicago, where the patient was first treated, have given permission to report the case.

management, the development of a rapid labored respiration on Sept. 30 led the attending pediatrician to administer 0.36 Gm. of sulfathiazole in three doses of 0.12 Gm. The respiratory difficulty, however, increased in severity in spite of the treatment, and an area of bronchial breathing in the left upper chest developed, accompanied by a bloody nasal discharge.

The baby was admitted to the Children's Memorial Hospital on Oct. 1, 1941, where his first blood count revealed 13,500 leucocytes with only 38 polymorphonuclear granulocytes. His temperature remained normal. Respiration was fast and labored, and the baby appeared cyanotic. Blood cultures were negative, and cultures of the blood-streaked stools yielded only *Bacillus coli* and nonhemolytic streptococci. The child did poorly in oxygen but responded readily to steam inhalations and four small transfusions from the father. A questionable area of consolidation corresponding to the region where the bronchial breathing had been heard was demonstrated on an x-ray film. Sulfathiazole medication was begun in doses of 1.9 gr. every six hours. A repeat x-ray showed a clear chest, but the thymus was thought to be somewhat enlarged, for which the patient was given 156 r. units on Oct. 9. On that day the infant was discharged from the hospital with a leucocyte count of 12,000. During his hospitalization he had received a total of 2.55 Gm. of sulfathiazole.

At home the baby's color remained good, but his nose continued to discharge bloody mucus, and his respirations were still of a "snorting" nature. Two subsequent blood examinations (Oct. 21 and 22) showed total leucocyte counts of 6,200 and 5,800, respectively, and the granulocytes were present in the amount of 48 per cent on each occasion. On Oct. 23 he received a second dose of x-ray therapy and was admitted to the Children's Memorial Hospital again because of the nasal condition.

Physical examination on this second admission revealed only the persistent presence of dry crusts and bloody discharge in the nose. The clinical impression was always that the difficulty was primarily due to obstruction, since it was always relieved by removal of the crusts; no other therapy was effective. On Oct. 25 a harsh systolic murmur at the apex was heard for the first time; this led to the assumption that the child had congenital heart disease, which might account for his ruddy color. The child was placed in a steam room for six days and was given a total of 0.803 Gm. of sulfathiazole. At this time his leucocyte count was 5,600, with 48 per cent polymorphonuclear granulocytes. On Oct. 29 the patient was transferred to the University of Chicago Clinics.

Examination revealed a well-nourished and well-developed male infant with a generally ruddy color of the skin which, upon vigorous crying, tended to become cyanotic. A loud systolic murmur was heard all over the precordium, being most pronounced at the apex and along the left border of the sternum. The liver was palpable one fingerbreadth below the right costal margin, and the tip of the spleen could also be felt. Both nostrils were completely filled with hard bloody crusts. Otherwise the examination was essentially negative. Since both the electrocardiogram and roentgenologic examinations were entirely normal, attention was chiefly directed to his nasal condition. The hard crusts were removed daily (Dr. Lindsay), as they would re-form each day. Some of the crusts were so large as to form almost a complete cast of the nasal cavity, and the condition was thought to be a type of atrophic rhinitis. The blood count at this time disclosed 10,150 leucocytes, of which 48 per cent were neutrophilic granulocytes.

Because of a daily rise in temperature up to 38° C. or higher, the posterior nasopharynx was examined under ether anesthesia on Nov. 7 for uncovering a possible focus of infection. No adenoid pad was observed; a small amount of free pus was seen in the nasopharynx, but no actual source of infection could be discovered. During the night following this examination the child developed an enor-

mous inflammatory swelling of the submaxillary glands, especially of the right side, from which pus could be expressed into the mouth. The temperature rose to 39.8° C. Sulfathiazole, in doses of 0.1 Gm. every four hours after the initial dose of 0.2 Gm.,

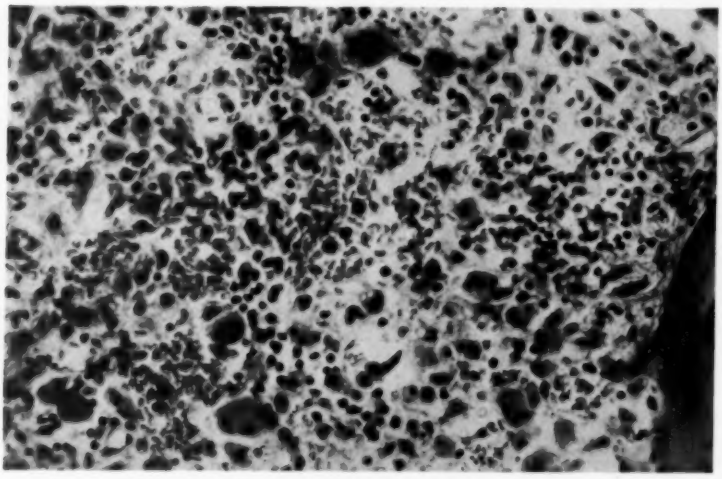

Fig. 1.—Photomicrograph of a section from the rib marrow, showing complete absence of mature granulocytes, hyperplasia of the reticular elements, and degenerating megakaryocytes. Hematoxylin-eosin stain. Magnification ×420.

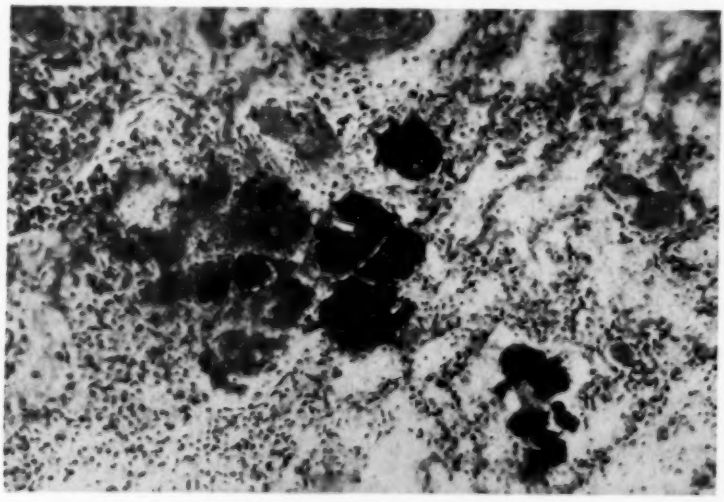

Fig. 2.—Photomicrograph of a section from the lung, showing large clusters of microorganisms in the pulmonary parenchyma. Hematoxylin-eosin stain. Magnification ×255.

was then given until a total of 0.6 Gm. was reached. Then, twelve hours after the first doses and in the space of two hours, the infant developed large bullae on each buttock and on the left foot. These lesions were extremely superficial, measuring from 0.5 to 3.0 cm. in diameter, and the intervening skin appeared to be

entirely normal. The sulfathiazole was discontinued at once, and sulfadiazine in the same dosage was substituted. By the morning of the following day, when 0.4 Gm. of sulfadiazine had been given, the baby's condition became much worse; many new bullae appeared on feet, hands, and chest. Culture of the fluid aspirated the day before from the bullae was sterile. All sulfonamides were stopped, and the sites of broken bullae were treated with gentian violet. Healing began at once.

The following day the bullae were healing well, and no new ones appeared, but slight exfoliation of the chest had begun. Some difficultiy in swallowing and moderate diarrhea developed on the same day, but otherwise there were no new findings. By the morning of Nov. 11 exfoliation of the chest and back had become quite extensive. Furthermore, the baby's mouth was found to be almost filled with a white cheesy material, from which pure cultures of *Monilia albicans* were obtained (Dr. S. Becker). Local therapy was begun with gentian violet, but the baby's general condition became progressively worse. A marked respiratory difficulty seriously interfered with bottle feeding. Because of the mouth lesions, it was not advisable to pass a stomach tube; the diarrhea precluded rectal administration of fluids, and the skin involvement was so widespread as to permit only very small amounts of subcutaneous fluids. On this day the blood leucocytes were 7,150, with 35 per cent granulocytes.

In another twenty-four hours the child's condition was grave. The swelling of the salivary glands had somewhat diminished, but all other symptoms were aggravated. A blood examination early in the morning revealed a leucocyte count of 3,150, with scant numbers of polymorphonuclear granulocytes, while later in the afternoon the blood leucocytes dropped to 2,000, with a complete disappearance of granulocytes. Pentnucleotide therapy was instituted at once, and a transfusion of 60 c.c. of father's blood was given, but with only a slight improvement. During the night the child seemed to rally somewhat, but at 6:15 A.M. on Nov. 13 he suddenly expired. Necropsy was performed (Dr. P. R. Cannon) three hours after death. The anatomic diagnosis (gross and microscopic) was as follows: Acute pulmonary edema and hemorrhagic bronchopneumonia; desquamative and bullous dermatitis; acute hemorrhagic and fibrinous glossitis, pharyngitis, and esophagitis (monilial infection); acute hyperplasia of the spleen; subacute inflammation of the right submaxillary gland; parenchymatous degeneration of the liver, kidneys, and myocardium; granulocytopenia of the bone marrow with degeneration of the reticular cells and edema (maturation arrest); lymphorrhexis in the thymus, lymph nodes, spleen, and bone marrow.

Sections of the bone marrow, stained with hematoxylin and eosin, showed practically a complete absence of granulocytes. The reticular cells were swollen and many of them contained hyperchromatic, irregularly shaped, and, at times, pyknotic nuclei. The reticular tissues were edematous. Megakaryocytes exhibited nuclear fragmentation, and the nuclei of many of the lymphocytes were distorted, fragmented, or pyknotic. Sections from the sternum, rib, vertebra, and tibia were similar, indicating a general "maturation arrest" of the granulocytic series (Fig. 1).

In the lungs, bacterial colonies consisting entirely of cocci were present in and near bronchi. Practically no granulocytes were present around the bacterial masses or elsewhere in the edematous lungs, and no monilial organisms were seen within alveoli or around the bacterial masses (Fig. 2).

The characteristic lesions of thrush were present in the mouth, throat, and esophagus, with the monilial organisms readily seen. Colonies of cocci were also present on the denuded epithelial surfaces, but practically no granulocytes were present around them.

COMMENT

The pathogenesis of granulocytopenia, and particularly of agranulocytosis associated with sulfonamide therapy, is usually explained on the basis of either intoxication or sensitization. In spite of the general impression that sulfathiazole is less toxic than other members of the sulfonamide group, it is important to remember that a small percentage of patients treated with this drug may have an idiosynerasy to it even in therapeutic doses. In all the recorded cases of fatal agranulocytosis following sulfathiazole therapy, the dosage administered has been relatively large, supporting the conception that the overdosage of drugs containing benzene rings is etiologically related to the development of agranulocytosis. In the present case, however, the dosage of sulfathiazole employed was certainly within the safe therapeutic range, the total amount given being only 4.153 Gm. over a period of five weeks, with frequent interruptions. Unfortunately, the actual sulfathiazole concentrations in the blood of this patient, as well as the amounts of the drug excreted in the urine, were never determined. It is reasonable to suppose, however, that considering the small total dosage of sulfathiazole administered, the level of the drug concentration in the blood could not have been abnormally high. If the granulocytopenia were due to drug intoxication, the only possible explanation would lie in the probable idiosynerasy of this child to sulfonamide even in therapeutic dosage. Leser[6] stated that the dosage of sulfonamide capable of producing agranulocytosis varies widely, in one series the average being 53 Gm., and the extremes, 18 to 19 Gm. A cumulative toxic effect of the drug is unlikely, since the sulfonamides in general are rapidly eliminated through the urinary tract.

Another mechanism whereby acute agranulocytosis, due to high degree of idiosynerasy in susceptible patient, may result from small amounts of the sulfonamides, is the phenomenon usually referred to as sensitization. Long, Haviland, Edwards, and Bliss[7] commented on the instances of severe toxic symptoms of the sulfonamides when the drugs are given a second time in the same patient. For this reason, these authors suggested that if a patient gives a previous history of a toxic reaction to the drug, it is advisable first to employ a test dose of 0.3 Gm. and wait for twelve hours before cautiously beginning the course of therapy. That the sulfonamides are capable of inducing a sensitization phenomenon in susceptible subjects has been known for some time. Certain skin reactions, such as erysipeloid, scarlatiniform, morbilliform, pemphigoid, pustular and nodular eruptions, as well as severe and extensive exfoliative dermatitis, which may occur during the first two weeks of sulfonamide therapy, may be manifestations of either intoxication or sensitization. Purpuric and urticarial types of skin rash, which have been described as occurring in the course of sulfathiazole therapy, may be due to anaphylactoid reactions. Goodman and Levy[8] were the first to describe three instances of sulfanilamide toxicodermatosis, in

two of which a cutaneous scratch test gave positive reactions to the drug. Rogers[9] reported a case of definite sensitization to the drug, in which a flare-up reaction in areas that had previously been sensitized by some derivatives of the aminobenzene base (procain, tutocain, butyn and anesthesin) was noted upon administration of only 1.6 Gm. of sulfanilamide. In the patient studied by Goodman,[10] the skin manifested a specific hypersensitiveness to the substituted aniline base, para-amino benzoyl, which constitutes the nucleus of most drugs used as local anesthetics and to which the sulfonamides are related. Furthermore, in a case of fixed eruption observed by Loveman and Simon,[11] the eruption in the exact location was again elicited by giving not only sulfanilamide but azosulfamide as well. Goodman and Arthur[12] also reported a case of fixed eruption produced by sulfanilamide but which failed to show any reaction with sulfapyridine. Finally, Stephan Epstein[13] described both photoallergy and primary photosensitivity to sulfanilamide and studied the mechanism of this sensitivity. All these observations confirm the hypothesis that the sulfonamides are capable of inducing a state of hypersensitivity in a certain number of susceptible individuals. Hence in the case of our infant the presence of cutaneous manifestations occurring on remedication with small amounts of sulfathiazole presupposes a hypersensitivity to the drug which might have been a more important etiologic factor than simple intoxication.

A similar explanation may be applied to the changes which occur on the mucous surfaces of the body as a result of sulfonamide sensitization. In our patient definite changes were noted in the conjunctivae, especially marked during the terminal stage. Volini, Levitt and O'Neil[14] reported that the involvement of the conjunctivae occurred in association with four of the seven cases of cutaneous rash in their sulfathiazole-treated patients. The conjunctivitis in these instances was frequently very severe, with a bright red injection of the palpebral and bulbar conjunctivae, with edema of the eyelids and a seropurulent discharge. Some degree of photophobia was also present. These changes, as a rule, are of relatively short duration, and no permanent aftereffects have been observed.

The most important finding in our patient is the development of acute leucopenia with complete disappearance of granulocytes in the peripheral blood. Hematologically this was a case of true agranulocytosis, confirmed by autopsy findings. Due to the serious condition of the patient, it was not possible to perform a sternal marrow puncture before death, but smears made from marrow materials obtained at necropsy from the rib, femur, and vertebra revealed complete absence of granular leucocytes, and the peroxidase reaction was entirely negative. While only one case of complete recovery from acute agranulocytosis due to sulfathiazole medication has been reported (Pippin[3]), it is generally accepted that the condition usually terminates in death because of lack of a natural defense against the ravages of invading microorganisms.

In our patient, as in many typical instances of fatal agranulocytosis, the sections of the lungs and other tissues were found to contain numerous clusters of bacteria multiplying almost unchecked, due to the lack of mobilization of granular leucocytes. The histologic proof of maturation arrest in the granular leucocytes, called "granulocytic anakmesis" by Darling, Parker, and Jackson,[15] leads one to assume that the toxic effect of sulfathiazole on the bone marrow may be primarily that of interfering with certain biologic processes which enter into the elaboration of specific granules in the mature leucocytes. The majority of blood cells still present in the bone marrow in cases of acute agranulocytosis are immature myeloid elements which contain very few or none of the specific granules which give positive peroxidase reaction.

SUMMARY

A case of acute and fatal agranulocytosis with toxic dermal lesions produced by therapeutic doses of sulfathiazole, occurring in an infant 8 weeks old, is reported. At the time of this writing, no other cases of agranulocytosis following sulfathiazole medication in infants have been recorded, although instances of extreme granulocytopenia in children due to other members of the sulfonamide group have been observed. An experience of this sort re-emphasizes the necessity of exercising the utmost care in the therapeutic use of sulfathiazole in infants and children and of making frequent blood examinations as well as of determining the concentration of the drug in the blood. Despite the fact that absolute proof of this sulfonamide being the sole cause of agranulocytosis is lacking, the circumstantial evidence in our case strongly suggests that both intoxication by and sensitization to this drug played significant roles in the production of the toxicodermatosis as well as of the agranulocytosis which resulted in the death of the patient.

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MIXED IMMUNIZATION IN INFANCY AND CHILDHOOD

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SINCE Ramon[1] showed the fallacy of the German conception that immunization against any organism must be completed before starting immunization against any other organism, there have been many attempts to take advantage of the "antigenic synergy" which he postulated. While any combination is theoretically possible, for all practical purposes we are limited to combinations in prophylaxis against the three definitely accepted as of routine value in infancy: diphtheria, whooping cough, and tetanus. The combination of diphtheria toxoid and tetanus toxoid, both alum-precipitated, has been definitely shown[2] to result in higher antitoxic values against both organisms than single prophylaxis. The use of a combination of diphtheria toxoid, alum-precipitated, and whooping cough vaccine was stimulated by the animal experiments of Schütze,[3] which resulted in the clinical reports of Simon and Craster,[4] Sauer and Tucker,[5] Daughtry-Denmark,[6] and Kendrick.[7] The combination of clear diphtheria toxoid, alum-precipitated tetanus toxoid, whooping cough vaccine, and whooping cough toxin was first reported by us[8] recently, and an alum-precipitated combination of diphtheria and tetanus toxoids and whooping cough vaccine has been reported by Daughtry-Denmark,[6] and concurrent immunization by Miller and Saito.[9]

Since at the present moment the practicing physician has no guide in this welter of conflicting claims of which types of mixed immunization are best, it was thought desirable to do a controlled study, comparing not only the antigenicity of the various components, but also the reactions produced by the various types of mixed immunizations in infancy and childhood.

MATERIAL

The children were referred to the clinic by the hospital infant hygiene clinic and by local social service agencies. Fifty well children from 6 months to 8 years of age who had never been seriously or acutely ill were given injections at monthly intervals, according to the schedules detailed in Tables I to VII. Distribution according to color was forty-two white and eight Negro; according to sex, twenty-three male and twenty-seven female. All were clinic patients; all were given an injection monthly. The mothers were given mimeographed direction sheets and were instructed in how to take and record the rectal temperatures of the children three times a day for the forty-eight-hour period following each injection and how to measure and record the size of the area of inflammatory reaction on the arm in inches.

From the Immunization Clinic of the National Councilhouse.

TABLE I

Serologic Results and Reactions From Triple Immunization of Infants by Three Monthly Injections of Pertussis Vaccine (Total, 80 Billion), Followed by Three Monthly Injections of Clear Diphtheria Toxoid (Total, 2.5 c.c.), and Terminating With Two Injections of Alum-Precipitated Tetanus Toxoid (Total, 1.0 c.c.) at Two-Month Intervals (Tests Three Months Afterward)

SERUM NO.	AGE AT START (MO.)	SCHICK TEST	DIPHTHERIA ANTITOXIC LEVEL IN BLOOD (UNITS PER C.C.)*	TETANUS ANTITOXIC LEVEL IN BLOOD (UNITS PER C.C.)†	WHOOPING COUGH		REACTIONS	
					AGGLUTINATION TEST	COMPLEMENT FIXATION TEST	FEVER (DEGREES)	LOCAL (INCHES)
15	12	Neg.	+0.02	+0.1	4+	Anticomp.	100	0.5
42	8	Neg.	+0.1	0.2	Neg.	2+	99	0.0
50	22	Neg.	0.02	0.2	4+	Anticomp.	99	0.0
54	10	Neg.	+0.02	0.003	Neg.	2+	101	1.0
3A	17	Neg.	0.05	0.05	4+	4+	99	0.0
5A	28	Pos.	0.005	0.2	4+	q.n.s.‡	99	0.5
6A	19	Neg.	0.2	0.2	4+	1+	100	1.0
7A	19	Neg.	0.1	0.1	2+	q.n.s.	100	1.0
8A	18	Neg.	0.05	0.05	4+	2+	100	0.5
10A	20	Neg.	0.1	0.2	4+	4+	100	0.5
Averages		90% Pos.	+0.07	+0.13	3.0+	2.5+	99.6	0.35

*The antitoxic level diphtheria titrations were made on all serum samples at the following levels: 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 units.

†The antitoxic level is expressed in units (American) per cubic centimeter of blood serum. Tetanus antitoxin titrations were made on all serum samples at the following levels: 0.003, 0.01, 0.017, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 units.

‡q.n.s., Not sufficient serum.

TABLE II

Serologic Results and Reactions From Triple Immunization of Infants by Three Monthly Injections of Pertussis Vaccine (Total, 80 Billion), Followed by Two Injections Two Months Apart of Alum-Precipitated Diphtheria-Tetanus Toxoid (Total, 2.0 c.c.), (Tests Three Months Afterward)

SERUM NO.	AGE AT START (MO.)	SCHICK TEST	DIPHTHERIA ANTITOXIC LEVEL IN BLOOD (UNITS PER C.C.)	TETANUS ANTITOXIC LEVEL IN BLOOD (UNITS PER C.C.)	WHOOPING COUGH		REACTIONS	
					AGGLUTINATION TEST	COMPLEMENT FIXATION TEST	FEBRILE (DEGREES)	LOCAL (INCHES)
31	9	–	+0.5	+1.0	4+	Anticomp.	100	1.0
34	7	–	1.0	0.2	3+	2+	99	0.5
36	20	–	0.5	0.2	3+	3+	100	0.5
37	18	–	0.5	0.2	–	Anticomp.	100	0.0
41	9	–	+0.5	+0.1	4+	1+	99	0.0
56	6	–	1.0	0.2	4+	Anticomp.	101	0.5
100A	16	–	2.0	1.0	4+	4+	99	0.0
101A	22	–	0.05	0.5	4+	4+	99	0.0
102A	18	–	0.022	1.0	4+	Anticomp.	101	1.0
103A	16	–	0.2	0.5	2+	4+	99	0.0
Averages		100%	+0.63	+0.49	3.2+	3.0+	99.7	0.25

The prophylactic combinations shown in Tables I to VII were injected intramuscularly into an area of the upper part of the arm washed with alcohol only. Three months after the completion of the last injection, a Schick test was done and blood was withdrawn for serum titration. The serum titrations* employed were as follows:

1. The diphtheria antitoxic titer in the blood serum was estimated by Jones and Moss's[10] modification of Fraser's intracutaneous test on rabbits.

2. The tetanus antitoxic titer in the blood stream was estimated by Jones and Moss's[11] modification of the standard guinea pig test.

3. Pertussis agglutination was judged by the rapid agglutination test[12] and recorded as from negative to 4 plus.

4. Pertussis complement fixation was estimated by the method of Powell and Jamieson.[13]

Table I shows the reactions, both general and local, and the serologic results three months after the completion of the immunization of infants by three monthly injections of *Hemophilus pertussis* vaccine (20 billion to the cubic centimeter; total dose, 80 billion), followed by three monthly injections of clear diphtheria toxoid (total, 2.5 c.c.) and terminating with two injections, two months apart, of alum-precipitated tetanus toxoid (total, 1.0 c.c.). Experimental results in these children, who did not undergo mixed or combined immunization, are inserted in order that a comparison may be made between the ad seriatim, or one after another, immunization to which they were subjected and the various combinations detailed below. The *serologic results,* three months after the last injection, show an average level of diphtheria antitoxin of plus 0.07 unit per cubic centimeter, an average level of tetanus antitoxin of plus 0.13, an average pertussis agglutination of 3.0 plus, and an average pertussis complement fixation of 2.5 plus. The reactions resulted in an average temperature of 99.6° F., with 0.35 inch of induration.

Table II shows the reactions and serologic results of the immunization of infants by three monthly injections of *H. pertussis* vaccine (20 billion to the cubic centimeter; total dose, 80 billion), followed by two injections two months apart of alum-precipitated diphtheria-tetanus toxoid (total, 2.0 c.c.). The serologic results show an average level of diphtheria antitoxin of plus 0.63 unit per cubic centimeter, an average level of tetanus antitoxin of plus 0.49 unit per cubic centimeter, an average pertussis agglutination of 3.2 plus, and an average complement fixation of 3.0 plus. The febrile reaction averaged 99.7° F. and the area of induration, 0.25 inch.

Table III shows the reactions and serologic results from the immunization of infants by three monthly injections of combined alum-precipitated diphtheria toxoid-pertussis vaccine, followed by two injections at

*All serologic work was done by Dr. H. M. Powell and Dr. F. G. Jones of the Lilly Research Laboratory. I am indebted to Dr. W. A. Jamieson of Eli Lilly and Co. for a very generous supply of materials.

TABLE III

Serologic Results and Reactions From Triple Immunization of Infants by Three Injections of Diphtheria-Pertussis Vaccine, Alum-Precipitated (Total, 3.0 c.c.) Followed by Two Injections at Two Months Apart of Tetanus Toxoid, Alum-Precipitated (1.03 c.c., Total) (Tests Three Months Later)

SERUM NO.	AGE AT START (MO.)	SCHICK TEST	DIPHTHERIA ANTITOXIC LEVEL IN BLOOD (UNITS PER C.C.)	TETANUS ANTITOXIC LEVEL IN BLOOD (UNITS PER C.C.)	WHOOPING COUGH		REACTIONS	
					AGGLUTINATION TEST	COMPLEMENT FIXATION TEST	FEBRILE (DEGREES)	LOCAL (INCHES)
3	72	–	0.2	0.2	4+	3+	101	1.5
5	8	+	+0.002	+0.003	3+	q.n.s.	100	1.5
6	6	–	+0.002	+0.003	1+	q.n.s.	101	1.5
7	9	+	+0.002	+0.003	3+	4+	100	1.5
12	7	–	1.0	0.1	4+	4+	102	Abscess
18	13	–	0.2	0.2	–	1+	102	2.5
23	7	–	1.0	0.2	3+	A.C.	102	2.5
24	8	–	q.n.s.	+0.003	3+	A.C.	101	2.0
25	11	–	+0.02	0.1	–	A.C.	100	1.5
32	7	–	+0.002	0.1	4+	1+	100	1.5
Averages		80%	+0.27	+0.09	2.5+	2.6+	100.9	1.7 plus one abscess

TABLE IV

Serologic Results and Reactions From Triple Immunization of Infants by Receiving Three Monthly Injections of Pertussis Vaccine (Total, 80 Billion) and Simultaneously Two Injections of Diphtheria-Tetanus Toxoid, Alum-Precipitated (Total, 2.0 c.c.), Two Months Apart in the Other Arm (Tests Three Months Afterward)

SERUM NO.	AGE AT START (MO.)	SCHICK TEST	DIPHTHERIA ANTITOXIC LEVEL IN BLOOD (UNITS PER C.C.)	TETANUS ANTITOXIC LEVEL IN BLOOD (UNITS PER C.C.)	WHOOPING COUGH		REACTIONS	
					AGGLUTINATION TEST	COMPLEMENT FIXATION TEST	FEBRILE (DEGREES)	LOCAL (INCHES)
19	8	–	0.05	0.5	–	A.C.	99	0.5
21	8	–	0.1	0.2	4+	A.C.	102	1.0
22	8	–	0.1	0.1	3+	A.C.	101	1.0
26	7	–	+0.5	0.2	4+	1+	101	1.0
27	9	–	0.5	0.5	4+	1+	100	1.0
30	7	–	0.5	+0.5	3+	1+	99	1.0
44	9	–	0.1	0.1	3+	2+	102	1.0
49	7	–	0.2	0.2	3+	4+	99	1.0
52	11	–	0.02	0.2	3+	4+	101	0.5
53	9	–	0.2	0.05	1+	2+	101	0.5
Averages		100%	+0.23	+0.27	2.8+	2.1+	100.5	0.85

two-month intervals of tetanus toxoid, alum-precipitated. The serologic results show an average level of diphtheria antitoxin of plus 0.27 unit per cubic centimeter, an average of tetanus antitoxin of plus 0.09 unit per cubic centimeter, an average pertussis agglutination of 2.5 plus, and an average pertussis complement fixation of 2.6 minus. The febrile reaction averaged 100.9° F., and the area of induration, 1.7 inches, with one case eventuating in a typical "cold," alum abscess.

TABLE V

Serologic Results (Three Months Afterward) and Reactions From Triple Immunization of Infants by Receiving Three Monthly Doses (0.2 c.c., 0.3 c.c., and 0.5 c.c.) of Pertussis Vaccine, Diphtheria Toxoid, and Tetanus Toxoid, Alum-Precipitated Combination (Total Pertussis, 40 Billion; Diphtheria, 36 L F units; and Tetanus, 30,000 M. L. D.)

SERUM NO.	AGE AT START (MO.)	SCHICK TEST	DIPHTHERIA ANTITOXIC LEVEL IN BLOOD (UNITS PER C.C.)	TETANUS ANTITOXIC LEVEL IN BLOOD (UNITS PER C.C.)	WHOOPING COUGH		REACTIONS	
					AGGLUTINATION TEST	COMPLEMENT FIXATION TEST	FEBRILE (DEGREES)	LOCAL (INCHES)
16	24	–	0.2	0.2	2+	1+	101	1.5
17	96	–	0.05	0.2	3+	4+	100	1.0
33	8	–	0.2	0.5	4+	A.C.	102	1.0
45	7	–	0.2	0.2	1+	1+	100	1.0
47	8	–	+0.5	1.0	–	4+	99	0.5
48	8	–	2.0	0.5	4+	2+	101	1.0
51	7	–	0.5	0.2	4+	4+	100.5	1.5
63	72	–	0.007	1.0	1+	4+	99	0.5
55	11	–	0.5	0.1	1+	2+	101	1.0
2	7	–	0.5	0.2	4+	4+	103	Abscess
Averages		100%	+0.47	+0.41	2.4+	2.9+	101.6	0.9 plus one abscess

Table IV shows the reactions and serologic results from the immunization of infants by three monthly injections of pertussis vaccine and simultaneously two injections of diphtheria-tetanus toxoid, alum-precipitated, at two-month intervals, in the other arm. The serologic results show an average level of diphtheria antitoxin of plus 0.23, an average tetanus antitoxin of plus 0.28 unit per cubic centimeter, an average pertussis agglutination of 2.8 plus, and an average pertussis complement fixation of 2.1 plus. The febrile reaction averaged 100.5° F., and the local reaction, 0.85 inch.

Table V shows the reactions and serologic results from the immunization of infants by three monthly doses of a combined, alum-precipitated pertussis vaccine, diphtheria toxoid, and tetanus toxoid. The serologic results show an average level of diphtheria antitoxin of plus 0.47 unit per cubic centimeter, an average tetanus antitoxin of plus 0.41, an average pertussis agglutination of 2.4 plus, and an average pertussis complement fixation of 2.9 minus. The febrile reactions averaged 101.6° F. and the local reaction 0.9 inch, with one case eventuating in a typical, "cold," alum abscess.

Table VI is a recapitulation of the average findings in the five series of ten children each receiving this triple immunization by the five methods specified in Tables I to V. I have also incorporated, for purposes of comparison, the results with seventy-eight children previously reported (by me)[9] after having been injected with mixtures of clear diphtheria toxoid, alum-precipitated tetanus toxoid, pertussis vaccine, and pertussis toxin.

TABLE VI

Comparison of Serologic Results and Reactions From Triple Immunization of Infants by Various Methods (Average Figures Compiled From Tables I-V) (Tests Three Months Afterward)

METHODS TESTED	NUMBER OF CASES	SCHICK TEST (NEGATIVE IN %)	DIPHTHERIA ANTITOXIN LEVEL IN BLOOD (UNITS PER C.C.)	TETANUS ANTITOXIN LEVEL IN BLOOD (UNITS PER C.C.)	WHOOPING COUGH		REACTIONS	
					AGGLUTINATION TEST	COMPLEMENT FIXATION	FEVER (DEGREES)	LOCAL (INCHES)
(1) Three injections of clear diphtheria toxoid and two injections of alum-tetanus toxoid and three injections of pertussis vaccine given separately, ad seriatim	10	90.0	0.07	+0.13	3.0+	2.5+	99.6	0.35
(2) Three injections of pertussis, followed by two of diphtheria-tetanus toxoid, alum-precipitated	10	100.0	+0.63	+0.49	3.2+	3.0+	99.7	0.25
(3) Three injections of diphtheria-pertussis vaccine, alum-precipitated, followed by two of tetanus toxoid, alum-precipitated	10	80.0	+0.27	+0.09	2.5+	2.6+	100.9	1.7 and one abscess
(4) Simultaneous, three injections of pertussis vaccine in one arm and two of alum-precipitated diphtheria-tetanus toxoid in the other	10	100.0	+0.23	+0.27	2.8+	2.1+	100.5	0.85
(5) Alum-precipitated combination of pertussis vaccine, diphtheria toxoid, and tetanus toxoid in three monthly doses	10	100.0	+0.47	+0.41	2.4+	2.9+	101.6	0.9 and one abscess
(6) Clear diphtheria toxoid, alum-precipitated tetanus toxoid, pertussis vaccines, and pertussis toxin in five monthly doses as previously reported by author[9]	78	100.0	+0.1	+0.5	3.9+	3.8+	101.5	1.0

DISCUSSION

The usual method of immunizing infants and children has been to administer three injections, totaling 80 billion of pertussis vaccine, at monthly periods, followed by three monthly injections of clear diphtheria toxoid (total, 2.5 c.c.) and completed by two injections of alum-precipitated tetanus toxoid at two-month intervals (total, 1.0 c.c.). The results for diphtheria immunity noted in Table I (90 per cent negative for the Schick test and an average diphtheria antitoxin level of 0.07 unit per cubic centimeter) agree fairly well with those found in the literature[14, 15] for prophylaxis with three doses of fluid diphtheria toxoid; the tetanus antitoxic level (0.1303 unit per cubic centimeter) agrees with those previously reported for two doses of alum-precipitated toxoid,[16, 17] and whooping cough agglutination tests and complement fixation are consistent with those reported by Weichsel, Cohen, and me[18] and Mishulow.[19] In this discussion of alternate methods of immunization, these figures, therefore, will be used as the basis for comparison.

Since there is some theoretical objection to statistical evaluation based upon comparisons of the average antitoxic titers achieved by various methods of prophylaxis, the results of serum titrations for diphtheria antitoxin and tetanus antitoxin have been arranged in a table of antitoxin levels according to the various methods tested here (Table VII). For the sake of ready comparison, figures from some of the work in the literature are also incorporated in Table VII.

The use of three injections of combined alum-precipitated diphtheria toxoid-pertussis vaccine, as advocated recently,[3-7] followed by two injections two months apart of tetanus toxoid, alum-precipitated, resulted in a 80 per cent negative reaction to the Schick test, with an average antitoxin level for diphtheria of 0.27 unit per cubic centimeter, but analysis of the antitoxin level (Table VII) shows 44.4 per cent below 1/250 unit per cubic centimeter, the "safe antitoxic level."* This agrees substantially with Daughtry-Denmark's[6] work. The average tetanus antitoxic level was 0.09 unit per cubic centimeter with 40 per cent falling below the "safe antitoxic level."† Whooping cough agglutination averaged 2.5 plus, and complement fixation, 2.6 plus. In addition, reactions from this method of combined immunization were definitely more severe than with single prophylaxis, the average temperature after injection reaching 100.9° F., and the average area of induration, 1.7 inches, with one case resulting in a sterile abscess. All the workers with this combination[3-7] report the occasional occurrence of sterile abscesses after injection, and, following Bell,[20] attribute them

*"Safe antitoxic level" for diphtheria is considered as at least $\frac{1}{250}$ unit per cubic centimeter. It is generally conceded that there is no absolute correlation between a negative Schick test and an antitoxic level below $\frac{1}{250}$ unit per cubic centimeter. For practical purposes, a negative Schick test is considered the criterion of safety.

†"Safe antitoxic level" for tetanus is considered by most authors to be 0.1 unit per cubic centimeter, the level generally attained by the injection of 1,500 units of tetanus antitoxin.

TABLE VII

METHOD	PERCENTAGE OF SUBJECTS WITH DIPHTHERIA ANTITOXIN LEVEL OF:							PERCENTAGE OF SUBJECTS WITH TETANUS ANTITOXIN LEVEL OF:				
	NO. CHILDREN	<1:250	>1:250 <1:100	>1:100 <1:50	>1:50 <1:10	>1:10 <1:2	>1:2 <10	NO. CHILDREN	0.01 OR UNDER	0.01 TO 0.1	0.1 TO 1.0	1.0 AND OVER
Table I: Three monthly injections of pertussis vaccine followed by three monthly injections of clear diphtheria toxoid followed by two injections of alum-precipitated tetanus toxoid at two-month intervals	10	0	10	50	0	40	0	10	10	20	70	0
Table II: Three monthly injections of pertussis vaccine followed by two monthly injections two months apart of alum-precipitated diphtheria tetanus toxoid	10	0	0	0	20	10	70	10	0	0	70	30
Table III: Three monthly injections of diphtheria-pertussis vaccine, alum-precipitated, followed by two injections, two months apart, of tetanus toxoid, alum-precipitated	9	44.4	0	0	11.1	22.2	22.2	10	40	0	60	0
Table IV: Three monthly injections of pertussis vaccine and simultaneously two injections of diphtheria tetanus toxoid, alum-precipitated, two months apart	10	0	0	0	20	50	30	10	0	10	90	0
Table V: Three monthly injections of pertussis vaccine, diphtheria toxoid, alum-precipitated, combination	10	0	0	10	10	30	50	10	0	0	80	20
Table VIII: Combined immunization*	32	3	0	6	0	91	0	44	0	6.8	72.8	20.4
Fraser and Halpern: Three doses of fluid diphtheria toxoid†	36	0	5	3	14	53	25	–	–	–	–	–
Daughtry-Denmark:												
(a) Combined diphtheria toxoid and pertussis	36	52.8	0	11.1	0	33.3	2.8	–	–	–	–	–
(b) Combined alum-precipitated pertussis, diphtheria and tetanus toxoid‡	39	74.6	0	0	15.4	7.7	2.3	36	63.9	22.2	13.9	0
Miller and Saito: Simultaneous-like, Table II§	–	–	–	–	–	–	–	22	0	0	45.5	54.5

*Lapin: Am. J. Dis. Child. 63: 225, 1942.
†Canad. Pub. Health J. 26: 476, 1935.
‡Am. J. Dis. Child. 63: 453, 1942.
§J. PEDIAT. 21: 37, 1942.

to injections having been given too superficially. This, I feel, is an error. Alum-precipitated toxoid, whether diphtheria or tetanus, causes necrosis when injected into human tissue in a certain percentage of cases; pertussis vaccine often causes severe induration. When both alum-precipitated toxoid and pertussis vaccine are injected in combination, a certain percentage of alum abscesses results no matter how deep the injection may be. Jones,[21] working with guinea pigs, has found that the combination of alum-precipitated toxoid and pertussis vaccine may produce necrosis.

The use of three injections of pertussis vaccine, simultaneously or concurrently with two injections of diphtheria tetanus toxoid, alum-precipitated, in the other arm, advocated by Miller and Saito,[9] resulted in a 100 per cent negative reaction to the Schick test, with an average antitoxic level for diphtheria of 0.23 unit per cubic centimeter, and analysis of the antitoxic levels (Table VII) shows 0 per cent below 1/250 unit. Miller and Saito[9] unfortunately did not do antitoxin titrations for diphtheria. The tetanus antitoxin average is 0.27 unit per cubic centimeter, with 10 per cent falling below the safe antitoxic level of 0.1 unit per cubic centimeter. This agrees fairly well with Miller and Saito's[9] results for twenty-two children, since none of their children fell below this "safe antitoxic" of 0.1 unit per cubic centimeter. However, they gave a third dose of tetanus toxoid, which probably accounts for their higher levels, especially with 54.5 per cent having antitoxin levels over 1 unit per cubic centimeter. At this, the reactions from this method of combined immunization were definitely more severe than with single prophylaxis, the temperature averaging 100.5° F. after injection and the average area of induration, 0.85 inch, but no sterile abscesses occurred. The fever (it seems to me) is the result of the summation of the reaction from pertussis vaccine and the combined alum-precipitated toxoids, since it was highest after the first and third injections. At this point note should be made of the viewpoint of Miller and Saito,[9] that, if high levels of tetanus antitoxin are secured, the boosting dose of toxoid on exposure may be dispensed with. The whole history of active immunization against tetanus is based on the assumption that there are poor producers who will need "boosting doses" at the time of exposure. It must be remembered also that the level of tetanus antitoxin slowly falls over a period of years. Therefore we can never be sure whether a safe antitoxic level is present at the time of exposure, perhaps years later. Unless antitoxic titrations are done over a period of years, a "boosting dose" on exposure should never be dispensed with.[8]

The use of three injections of combined pertussis vaccine, diphtheria toxoid, and tetanus toxoid, all three alum-precipitated together, advocated by Daughtry-Denmark,[6] resulted in a 100 per cent negative reaction to the Schick test, with an average diphtheria antitoxin level of 0.47 unit per cubic centimeter, and analysis of antitoxic levels (Table

[AM. J. DIS. CHILD. 63: 453, 1942.
J. PEDIAT. 21: 37, 1942.

TABLE VIII

Follow-up of Children Given Mixed Immunization of Diphtheria Toxoid, Tetanus Toxoid, Pertussis Vaccine, and Pertussis Vaccine in Five Monthly Doses, as Originally Reported by Lapin*

SERUM NO.	FIRST TEST	SECOND TEST	DIPHTHERIA			TETANUS			PERTUSSIS						REMARKS
									AGGLUT. TEST			COMP. FIX.			
			3 MO.	6 MO.	12 MO.	3 MO.	6 MO.	12 MO.	3 MO.	6 MO.	12 MO.	3 MO.	6 MO.	12 MO.	
100	√	×	×	×	2.0	×	×	1.0	×	×	4+	×	×	4+	Mouse protection could not be repeated
101	√	×	×	×	0.05	×	×	0.5	×	×	4+	×	×	4+	
102	√	×	×	×	0.002	×	×	1.0	×	×	4+	×	×		
	√														
103	6 mo.	×	×	0.2	×	×	0.5	×	×	2–	×	×	4+	×	
104	√	×	×	×	0.2	×	×	0.05	×	×	2+	×	×	4+	
105	×	√	0.1	–	0.1	0.2		0.1	4+	–	1+	4+	–	4+	
106	×	√	–	–	0.05	0.2		0.2	2+	–	4+	4+	–	4+	
107	×	√	–	–	0.5	0.2	–	0.1	2+	–	4+	4+	–	4+	
108	×	√	0.1	–	0.05	0.2	–	0.1	4+	–	4+	4+	–	4+	
301	×	√	0.1	–	0.1	0.2	–	0.1	4+	–	4+	4+	–	4+	
302	×	√	0.1	–	0.05	0.2	–	0.2	4+	–	4+	4+	–	4+	
303	×	√	0.1		0.5	0.2	–	0.2	4+	–	4+	4+	–	4+	
304	×	√	0.01	–	0.002	0.2	–	0.1	4+	–	4+	4+	–	4+	
305	×	√	0.1	–	0.002	0.2	–	0.1	4+	–	4+	4+	–	q.n.s.	
306	×	√	0.1	–	0.05	0.2	–	0.2	4+	–	4+	4+	–	4+	
307	×	√	0.002	–	0.005	0.2	–	0.5	4+	–	4+	4+	–	4+	Sibling contact O.K.
308	×	√	0.01	–	0.02	0.2		0.1	4+	–	3+	3+	–	4+	
309	×	√	0.1		0.1	0.2	–	0.1	4+	–	4+	4+	–	4+	
310	×	√	–	–	0.5	0.05	–	0.1	4+	–	4+	4+	–	4+	
311	×	√	0.1	–	0.1	0.2	–	0.2	4+	–	4+	4+	–	4+	Sibling contact O.K.
	√														
1	6 mo.	×	–	q.n.s.	–	–	q.n.s.	–	–			–	–	q.n.s.	
2	×	√	q.n.s.	–	0.01	0.2	–	0.01	2+		3+	4+	–	3+	
3	×	√	0.1	–	0.05	0.2	–	0.05	4+		4+	4+	–	4+	
	√														
4	6 mo.	×	–	0.1	–	–	0.2	–		4–	–	–	2+		
5	×	√	–	–	0.005	–	–	0.2			4+	–	–	q.n.s.	
6	×	√	–	–	0.2	–	–	0.2	4+		4+	–	–	1+	

*Am. J. Dis. Child. 63: 225, 1942.

VII) shows 0 per cent below 1/250 unit per cubic centimeter. This compares very favorably with the subjects in Daughtry-Denmark's thirty-nine cases, 74.6 per cent of whom had an antitoxic level below 1/250 unit per cubic centimeter. Her unfavorable results were due to the use of a combination (B5778) containing 20 L F units of diphtheria toxoid, 10,000 minimum lethal dose of tetanus toxoid, and 20 billion pertussis vaccine per cubic centimeter, in contrast to the preparation I used (B6337), which contains 36 L F units of diphtheria toxoid, 30,000 minimum lethal dose of tetanus toxoid, and 40 billion pertussis vaccine per cubic centimeter. The tetanus antitoxin average is 0.41 unit per cubic centimeter, with 0 per cent below the safe 0.1 unit per cubic centimeter level. Daughtry-Denmark's results in thirty-six subjects show 86.1 per cent falling below 0.1 unit per cubic centimeter. The reactions from this method of immunization were severe, the temperature averaging 101.6° F. after injection and the area of inflammation, 0.9 inch, with one sterile abscess. Objections to this method of triple immunization are similar to those given above for the combined diphtheria toxoid and pertussis vaccine. Whenever alum is mixed with pertussis vaccine, the summation of necrotizing properties is so great that a fair percentage of alum abscesses results.

The use of three injections of pertussis vaccine, followed by two injections of combined alum-precipitated diphtheria-tetanus toxoids (Table II) resulted in a 100 per cent negative reaction to the Schick test, and an average diphtheria antitoxic level of 0.63 unit per cubic centimeter, with 0 per cent falling below 1/250 unit per cubic centimeter. Both the average unitage and the distribution in high antitoxic levels are definitely superior to any of the other methods tested, as regards prophylaxis against diphtheria. The average tetanus antitoxic level was 0.49 unit per cubic centimeter, agreeing very well with Bigler and Werner's[2c] average of 0.50 unit per cubic centimeter, and 100 per cent had levels above the safe level of 0.1 unit per cubic centimeter. Reactions averaged 99.7° F. in temperature, with only 0.25 inch of inflammation, substantially the same as with single prophylaxis. In addition, in an experience of over 100 injections of the alum-precipitated combined toxoid, no alum abscess has occurred.

For the sake of comparison, the results with injections of clear diphtheria toxoid, alum-precipitated tetanus toxoid, pertussis vaccine, and pertussis toxin according to a five-month schedule previously published[8] are incorporated in Table VI. The low average antitoxic average for diphtheria (0.1 unit) serves to emphasize what I previously said[8] of the superiority of alum-precipitated diphtheria toxoid. Table VIII presents a follow-up of twenty-six of these seventy-eight cases. After twelve months, 13.0 per cent had fallen below the safe diphtheria antitoxic level of 1:250 unit per cubic centimeter, and 13.0 per cent had fallen below the safe tetanus antitoxin level of 0.1 unit per cubic centimeter. In contrast, the pertussis agglutination and complement

fixation tests remained just as high when tested after twelve months as after the original three-month period.

CONCLUSION

An attempt has been made to evaluate the different methods of combined or simultaneous immunization advocated for routine prophylaxis in infancy against diphtheria, tetanus, and whooping cough. Of a total of fifty children, ten were injected with pertussis vaccine, fluid diphtheria toxoid, and alum-precipitated toxoid in the usual ad seriatim procedure. While reactions were few, this produced the lowest diphtheria antitoxic titer, 0.07 unit per cubic centimeter, and next to the lowest tetanus antitoxic titer, 0.13 unit per cubic centimeter. Another ten children were given three injections of alum-precipitated combined diphtheria toxoid and pertussis vaccine, followed by two injections of alum-precipitated tetanus toxoid. This resulted in a slightly higher diphtheria antitoxin value (0.27 unit per cubic centimeter), a low tetanus antitoxic value of 0.09 unit per cubic centimeter, and severe febrile and inflammatory reactions with one alum abscess. Another ten children were given three injections of pertussis vaccine and concurrently (or simultaneously) two injections of alum-precipitated diphtheria-tetanus toxoid. This resulted in a fair diphtheria antitoxin value of 0.23 unit per cubic centimeter and tetanus value of 0.27 unit per cubic centimeter with some febrile reactions but no abscesses. Another ten children were given alum-precipitated pertussis vaccine, diphtheria, and tetanus toxoids, combined, in three injections. This resulted in the high diphtheria antitoxin average of 0.47 unit per cubic centimeter and tetanus antitoxin average of 0.41 unit per cubic centimeter, but febrile and inflammatory reactions were severe and one abscess resulted. The last group of ten children were given 3 injections of pertussis vaccine followed by two injections of alum-precipitated combined diphtheria tetanus toxoids. This resulted in the highest antitoxin averages of any group, 0.63 unit for diphtheria and 0.49 unit for tetanus. The febrile and inflammatory reactions were mild and no abscesses resulted.

If the results of this study are confirmed in larger groups, prophylaxis by three monthly injections of pertussis vaccine followed by two injections at two-month intervals of combined alum-precipitated diphtheria and tetanus toxoids is superior because of its antigenicity and also because of its freedom from reactions to the ad seriatim method now in vogue and to the other methods of combined immunization.

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1166 Grand Concourse

WHOOPING COUGH VACCINES

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EFFORTS to avoid local and systemic reactions with whooping cough vaccines are necessary in view of Ravenel's[1] and my own[2] reports of febrile reactions and pain at the local site within a few hours, in a large proportion of children injected, no matter which of the commercial vaccines is used. The aim of this work is to develop a vaccine of the highest possible immunizing power with the lowest possible reactions.

The local and systemic reactions after injection of whooping cough vaccine into susceptible infants show tremendous variations. In many children the injection of 2 c.c. of the 20 billion to the cubic centimeter vaccine is attended by no fever and no pain or swelling; in others, a dose of even 10 billion will produce moderate fever, an area of erythema two inches in diameter, and severe pain for several hours. Immediately after the prick of the needle, there seems to be little pain or redness, but at a period ranging from four to twenty-four hours after the injection, the child feels heat and throbbing, and the site becomes red and tender.

What constituent of the vaccine is responsible for the local and systemic reaction has been the subject of much dispute. Certain possibilities are as follows:

1. The chemical used to preserve the vaccine, i.e., phenol, formalin, merthiolate, etc. No work has been published showing the percentage of reactions after injections of vaccines made with these different preservatives.

2. The old question of "washed" or "unwashed" vaccines, which must be reviewed both from the standpoint of maintenance of antigenicity and avoidance of reactions. Sauer[3] had originally recommended recapturing the pertussis organisms in saline without washing on the ground that when pertussis organisms are washed, the filtrate produces an intracutaneous reaction in injection into human beings. This reaction, he felt, showed that washing removed some principle of importance in the immunology of whooping cough. The poor results in prophylaxis of the Cleveland group,[4] who used a vaccine made by washing with distilled water as compared with Kendrick and Eldering's[5] good results with a vaccine washed once in saline, have been attributed to solution in the wash water of some cell surface component essential in immune reactions. Although this was not confirmed by experimental work,[6] the use of unwashed vaccine has become traditional. The recent articles of Flosdorf and his group[7] on the components of the cell surface

From the Immunization Clinic of the National Council House.
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of *Hemophilus pertussis* have thrown much light on the whole problem. They showed there are at least two components of the cell surface: (a) a strongly antigenic, agglutinogenic component and (b) a poorly antigenic or toxic component. The antigenic, agglutinogenic component does not produce an intracutaneous reaction in immune subjects but the toxic, weakly antigenic component does. It should be possible, therefore, to devise a method of washing or otherwise modifying the methods of preparation of pertussis vaccines to minimize their toxic, reaction-producing component while preserving the antigenic agglutinogen, which produces no reactions.

3. The necessary incorporation of blood in the modified Bordet-Gengou medium. Sauer (Q. V.) has expatiated on the necessity of human blood in order to obviate possible foreign protein sensitization. Mishulow,[8, 9] on the other hand, presented figures to show that the amount of sheep's blood in her vaccine (0.00328 c.c.) was far too small to produce sensitization. However, in a recent case in which I used a sheep's blood vaccine and got a very severe reaction, I later found an intracutaneous reaction to sheep's serum strongly positive. The use of bovine blood seems to have obviated these difficulties, since no therapeutic serums are made from bovine blood. However, the possibility of reaction from the blood in the vaccine has led others to prepare a medium from which all blood or foreign proteins have been excluded but which retains the phase I character of *H. pertussis*. No vaccine prepared in this way has been used to test its antigenicity or its capacity clinically to protect against pertussis.

4. The skin-necrotizing quality of *H. pertussis* endotoxins, which theoretically, at least, are not destroyed by any washing or preservative. This skin-necrotizing quality has led to the use of the Döld test[10] as a measure of the potency of the cultures used and I have used it as a basis for judgment of the value of immune sera.[11]

In this paper, the problem was attacked by the following methods:

1. Injection of a suspension of living *H. pertussis* organisms in normal saline, three days old, in a concentration of 10 billion organisms to the cubic centimeter, three doses of 1 c.c. being given at monthly intervals. Since the suspension had not been heat-killed and no preservative had been used, reactions from it could be due only to the bovine blood in the original medium, to the exotoxin of *H. pertussis*, or to the endotoxin of *H. pertussis*. Although the dosage was much smaller than commonly employed with vaccines, the reaction with a suspension of living organisms was very severe, and in fact, resembled the intracutaneous necrosis of the Döld test in rabbits.

2. Injection of a "sensitized suspension." The suspension of living *H. pertussis* organisms in normal saline, in a concentration of 10 billion organisms to the cubic centimeter, three days old, was mixed with one-half its volume of hyperimmune pertussis human serum,[12] 1.5 c.c. at monthly intervals. Hyperimmune pertussis human serum has been

shown in rabbits to neutralize the skin-necrotizing effect of a live suspension of *H. pertussis*. By virtue of the fact that the vaccine is unwashed, it contains exotoxins, which are responsible, as shown by Flosdorf,[7] for the intracutaneous reactions. North[13] holds that hyperimmune pertussis serum neutralizes these toxins too; Flosdorf[14] has not found any significant neutralization.

3. Injection of a "sensitized vaccine." A vaccine of *H. pertussis* organisms, in a concentration of 15 billion organisms to the cubic centimeter, was mixed with one-half its volume of hyperimmune pertussis serum, allowed to stand for twenty-four hours, and then injected in dosages of 1.0 c.c. at monthly intervals. This vaccine had been grown on bovine blood and killed and preserved with merthiolate (1:10,000). Since the exotoxins and endotoxins are theoretically neutralized by immune serum, reactions could be due only to the bovine blood in the medium or the merthiolate.

4. A vaccine of *H. pertussis* organisms in a concentration of 10 billion to the cubic centimeter precipitated with alum,[15] filtrate poured off, and the alum precipitate resuspended in normal saline. Three injections of 10 billion organisms were given at monthly intervals.

TABLE I

SEROLOGIC TESTS THREE MONTHS AFTER THE LAST INJECTION

PREPARATION USED	SERUM NO.	AGE	COMPLEMENT FIXATION	RAPID AGGLUT.	AVERAGE REACTIONS	
					LOCAL (INCHES)	FEBRILE (DEGREES)
Suspension of living H. pertussis, 10 billion to the c.c., three doses of 1 c.c.	39	1 yr.	anticomp.	–	2	101
	60	5 yr.	3+	–	2	102
	62	1 yr.	2+	–	2	101
Sensitized suspension, 10 billion to c.c. mixed with half volume immune serum, three doses of 1.5 c.c.	10	2 yr.	3+	–	1	100
	20	7 mo.	2+	–	½	101
	29	1 yr.	2+	–	1	101
Sensitized vaccine 15 billion to the c.c. mixed with half volume immune serum, three doses of 1 c.c.	1	3 yr.	3+	1+	½	100
	4	15 mo.	4+	–	0	99.8
	8	2 yr.	4+	1+	0	99
	9	3 yr.	4+	3+	½	99
	11	2 yr.	4+	1+	0	100
	1	4 yr.	4+	3+	0	99
Alum-precipitated vaccine, 10 billion to the c.c., three doses of 1 c.c.	43	22 mo.	3+	–	½	100
	46	6 yr.	2+	–	½	100
	65	19 mo.	anticomp.	4+	1	102
	38	2 yr.	anticomp.	–	½	100
	14	5 yr.	anticomp.	–	½	100
	13		2+	–	0	99
Commercial vaccine, 10 billion to the c.c.: (a) Human 1.0-1.0-1.0	1A	6 mo.	3+	2+	½	99
	2A	7 mo.	2+	3+	0	99
	3A	6 mo.	3+	3+	½	99
(b) Bovine 1.0-1.0-1.0	4A	8 mo.	4+	3+	0	99
	5A	6 mo.	3+	4+	0	99
	6A	9 mo.	2+	4+	0	99
(c) Sheep 1.0-1.0-1.0	7A	6 mo.	3+	4+	½	99
	8A	9 mo.	2+	3+	½	100
	9A	7 mo.	4+	3+	½	100

5. Injections of vaccines prepared on (a) human blood (Sauer's), (b) bovine blood (Lilly's), and (c) sheep blood (Mishulow) in concentrations of 10 billion to the cubic centimeter, 1 c.c. being given at three monthly intervals. These injections were compared in order to determine which gave the greatest reaction.

6. Injections of minimal quantities of (a) human blood, (b) bovine blood, and (c) sheep blood in the concentrations figured by Mishulow. These injections were made in order to determine if the reactions were due to the blood. No reactions were discovered.

Serologic Procedure.—The antigenicity of the various vaccine suspensions used were tested by complement fixation[16] and agglutination reactions[17] on serum withdrawn from the children three months after the last injection.

Clinical Material.—We used a group of twenty-seven children, from 6 months to 6 years in age, fourteen male and thirteen female, drawn from a very cosmopolitan district of New York City. Printed directions given to the mothers asked them to judge the size of the reaction in inches and to take the child's temperature three times in the twenty-four hours following each injection.

The results of the study are incorporated in Table I, with complement fixation and rapid agglutination tests in parallel columns with "average reaction," both local and febrile. This is the average of reactions after all three injections, the local area of redness diameter being measured in inches and the temperature in degrees fahrenheit.

REACTIONS AND SEROLOGIC RESULTS

1. After injection of a suspension of living *H. pertussis* organisms in normal saline, three days old, in a concentration of 10 billion to the cubic centimeter, three doses of 1 c.c. at monthly intervals. Although serologic results were good (complement fixation, 1 plus in two of three, and agglutination tests negative in all three), especially since only 30 billion organisms are given instead of the customary 80 billion vaccine, the reactions were very severe. The general febrile reaction averaged 101.5° degrees and area of inflammation, about two inches in diameter; the pain lasted two or three days. Since this suspension had not been heat-killed nor had any preservative been used, the reaction was probably due either to the bovine blood in the original medium or to the exotoxin or endotoxin of *H. pertussis*. The living organism is a powerful producer of potent toxin and so biologically different from a vaccine that there may be other factors besides toxins responsible. The reactions were so severe that the use of living suspensions in an effort to immunize children had to be abandoned.

2. After injection of the "sensitized suspension" of living organisms. The three-day-old suspension of living *H. pertussis* organisms in normal saline, in a concentration of 10 billion to the cubic centimeter, was mixed

with one-half its volume of hyperimmune human serum, allowed to stand twenty-four hours, and then injected in dosages of 1.5 c.c. at monthly intervals for three injections. This resulted in a total of 30 billion organisms being injected. Serologic results are good (complement fixation, 1 plus in three of three cases and agglutination tests negative in all three). The general febrile reaction averaged 101° F. and the area of inflammation about one inch in diameter; the pain lasted one day. In discussion, it seems evident that the reactions were minimized by the admixture of human immune serum but not enough to make the use of "sensitized suspension" advisable. Regardless of theoretical considerations, this suggests that hyperimmune serum does not neutralize both the skin-necrotizing effect and the toxins of *H. pertussis.*

3. After injection of a "sensitized vaccine." A vaccine grown on bovine blood, killed and preserved with merthiolate in a concentration of 15 billion organisms to the cubic centimeter, was mixed with one-half its volume of hyperimmune human serum, allowed to stand for twenty-four hours, and then injected in three doses of 1.0 c.c. at monthly intervals. This resulted in dosage of 30 billion organisms being injected. Serologic results are good (complement fixation, 1 plus in six of six and agglutination tests, 1 plus in five of six). General reactions were very mild (100° average); local reaction, one-half inch; and fever, 101° F.

4. After injections of alum-precipitated vaccine, 10 billion to the cubic centimeter, three monthly doses of 1 c.c. Serologic results were good (complement fixation, 1 plus in three of six cases, anticomplementary in the other three, and agglutination, 1 plus in one of six cases), but reactions were moderately severe, with local areas averaging one-half inch and fever, 101° F.

5. After injections of vaccines prepared on (a) human blood, (b) bovine blood, and (c) sheep blood in concentrations of 10 billion to the cubic centimeter, in dosages of 1.0 c.c., at three monthly intervals. Serologic results were excellent in all cases (complement fixation, 1 plus and agglutination, 3 plus or 4 plus in each case of all nine tested, irrespective of the product), and reactions were mild.

6. The injection of 0.005 c.c. of blood, whether human, bovine, or sheep blood. This injection was not attended with any local or febrile reactions in six children into whom it was injected.

DISCUSSION

The limitations on the number of cases in each category made necessary by the restrictions on wartime research are such that no definite conclusions can be drawn from this work. Certain tentative conclusions may be made, but only further work with large series will test the validity of the results. These are as follows: (1) The type of blood used in preparing the vaccine is of no importance from a serologic view-

point (if human, bovine, or sheep), nor is the blood responsible for the reactions suffered in vaccine administration. (2) The preservative used is of no importance from the viewpoint of reactions, although it may be important for the preservation of antigenicity.[18] (3) The addition of one-half the volume of hyperimmune human serum to a living suspension does not obviate reactions altogether, but the addition of serum to a vaccine does seem to minimize reactions. Unfortunately, from the few cases available, it seems possible that the addition of the serum seems to have interfered with the antigenicity of the suspension or vaccine. Thus, it will be noted that serologic results after 30 billion of ordinary vaccine seem distinctly superior to the results of 30 billion of suspension or vaccine sensitized by the addition of hyperimmune serum. This may be only a temporary phenomenon, such as Ramon[19] speaks of in discussing his combined Active-Passive Immunization, or the quantity of serum may have been too large. It certainly seems questionable to sacrifice antigenicity to the reduction of reactions. (4) The use of alum-precipitated vaccine does not seem to have given better serologic results than ordinary vaccine, and the reactions were definitely more severe. No alum abscesses were present in this small group (six cases), but even the small percentage reported in the literature should make the physician guard against the use of alum vaccines except where markedly superior antigenicity is evident. For the same reason, the use of combinations of whooping cough vaccine with alum-precipitated toxoids is to be discouraged until further refinements are available.

Harrison, Franklin, and Bell,[20] Bell,[21] and Daughtry-Denmark[22] have used alum-precipitated pertussis vaccines. Only the work of Daughtry-Denmark is comparable. Giving a total of 20 billion alum-precipitated in three doses, she secured 87.1 per cent positive complement fixation, and with a total dose of 40 billion, secured 94 per cent positive complement fixation, as compared with 99 per cent positive complement fixation for 100 billion total dosage of the ordinary vaccine. Unfortunately, the complement fixation tests were done only one week after the completion of the immunization, as compared with three months after in this paper. Reference to Table I will show 100 per cent of all twenty-seven children showing positive complement fixation, but a much lower figure (55 per cent) as judged by the rapid agglutination test, which reaches its height much later than the complement fixation reaction.[22]

CONCLUSION

Living suspension, suspensions sensitized by the addition of immune serum, and alum-precipitated vaccines present no serologic advantage, but on the other hand produce more severe reactions than ordinary whooping cough vaccines. Neither the blood in the vaccine, nor the type of blood (whether human, bovine, or sheep), nor the preservatives used are responsible for the reactions with whooping cough vaccines. A

vaccine sensitized by immune serum gives definitely less severe reactions, but its antigenicity may be either delayed or impaired.

There is a great need for whooping cough vaccine in which the toxic, weakly antigenic component responsible for the reaction is kept down to a minimum and the agglutogenic, antigenic, nonreactive component preserved at a maximum level. Certain suggestions for the future can be made. If the growth from the fresh, phase I *H. pertussis* culture is suspended in a large quantity of saline and Berkefeld-filtered, it seems probable that all of the thermolabile and a large part of the thermostable toxins of *H. pertussis* will be destroyed in the process of filtration. If the Berkefeld-filtered vaccine no longer produces an intracutaneous reaction in rabbits, and vaccine injections produce a high agglutinin titer on injection into rabbits, the theoretical requisites for a better whooping cough vaccine discussed in this article will have been fulfilled. Such work is now under way.

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TREATMENT OF PAROXYSMAL AURICULAR TACHYCARDIA IN INFANCY AND CHILDHOOD

Report of a Case

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PAROXYSMAL auricular tachycardia in infants and children is sufficiently uncommon to warrant discussing a recurrent case in a 2½-year-old boy. Although both the etiology and diagnosis of this arrythmia have been well reviewed, there is little information relating to the treatment in young individuals. This is true not only in regard to the choice of the proper drug, but also in regard to suitable dosage in pediatric practice. It is this aspect which I intend to emphasize.

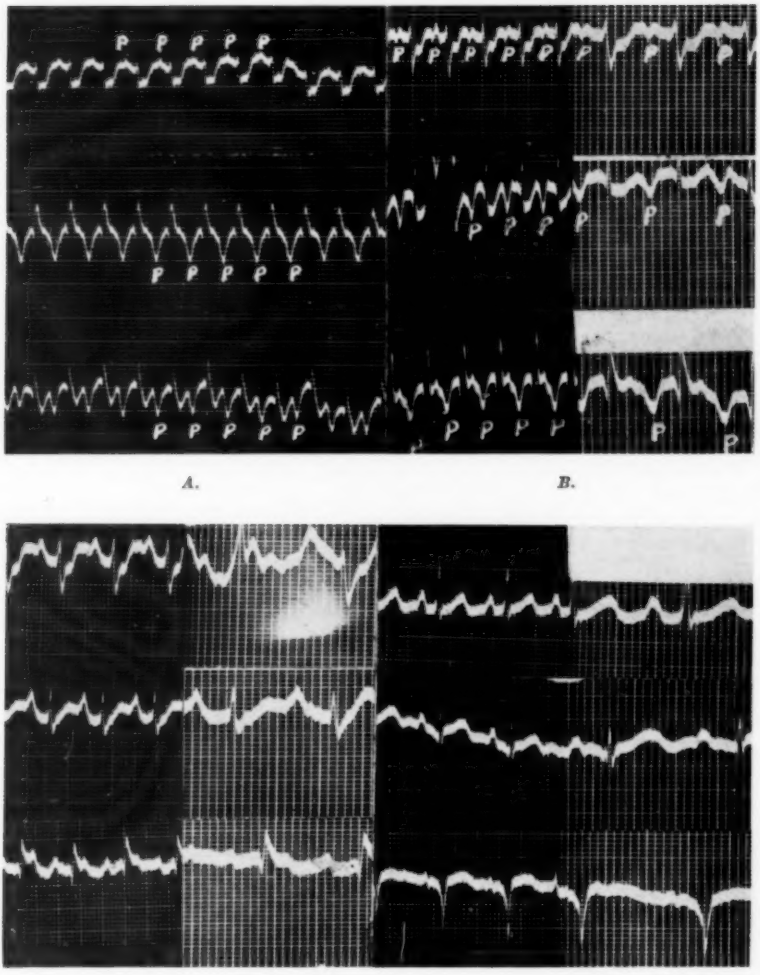
CASE REPORT

On March 6, 1941, E. K., a 2½-year-old boy, was admitted to the Brooklyn Hospital on the service of Dr. Harry A. Naumer. The chief complaint, according to the mother, was "a rapid heart" of about three hours' duration. The onset occurred suddenly when the child became slightly cyanotic after breakfast. This was approximately the fourteenth such attack since birth. The patient was alleged to have been a "blue baby" at birth. The previous episodes of tachycardia had been noticed intermittently during the ensuing two and one-half years. They were accompanied by varying degrees of cyanosis and orthopnea. On several such occasions the child was hospitalized at the Methodist-Episcopal Hospital where diagnoses of paroxysmal auricular tachycardia and auricular flutter with a one-to-one rhythm were made. Usually a concomitant upper respiratory infection or pneumonia was present. Apparently the only treatment which had been successful was digitalization plus control of the respiratory infection. The family history was interesting in that there was one sibling who was said to have died at an early age as a result of a congenital heart lesion of unknown type.

Examination on admission showed a well-developed and well-nourished young white boy in moderate distress. The child weighed thirty pounds. The temperature was 99.4° F. The patient was orthopneic. The radial pulse was indistinct, and the blood pressure was not obtainable. There was a malar flush and moderate cyanosis of the nailbeds and lips. The external jugular veins were full and pulsated synchronously with the apex beat. The superficial veins of the upper part of the abdomen and the chest were slightly dilated. No deformity of the chest or clubbing of the fingers or toes was noted. The pharynx was slightly injected. The apical impulse was diffuse, and there was no thrill. The left border dullness was at the anterior axillary line, and the right border of the heart could not be percussed beyond the sternum. The apical rate was 208 per minute and regular in rhythm and force. The heart sounds were of good quality and intensity. No murmurs were heard at this time. The lungs were clear to percussion and auscultation. The liver edge was felt one fingerbreadth below the costal margin and was not tender. The spleen was not palpated. No sacral or pretibial edema was present.

The sedimentation rate and the blood sugar were normal. The blood Wassermann reaction was negative. Urinalysis revealed no abnormalities. The hemoglobin was 84 per cent (Sahli), the red cell count was 4,500,000, and the leukocyte count was 24,600, with a normal differential count.

From the Department of Pediatrics of the Brooklyn Hospital, Brooklyn, N. Y.



A. *B.*

C. *D.*

Fig. 1.—Electrocardiograms taken during first admission. *A*, March 6, 1941, the day of admission. Auricular tachycardia with a rate of 208 per minute and right bundle branch block. The P-R interval is normal (0.12 sec.), and the QRS interval is prolonged (0.10 sec.) Right axis deviation, axis about plus 136°.

B, March 7, 1941, the day after admission. Auricular tachycardia with a rate of 200 per minute and right bundle branch block. The P-R interval is normal (0.128 sec.), and the QRS interval is prolonged (0.08 sec.). Right axis deviation, axis about plus 160°. (One-half of each tracing is taken with the speed of the camera doubled in this and the following tracings.)

C, March 10, 1941, after restoration of sinus rhythm. Sinus rhythm with rate of 125 per minute and right bundle branch block. P-R interval is prolonged (0.20 sec.), and the QRS interval is prolonged (0.10 sec.) Right axis deviation, axis about plus 170°.

D, March 13, 1941, the day before discharge. Sinus rhythm with rate of 100 per minute. Right bundle branch block has disappeared. The P-R interval is prolonged (0.24 sec.), and the QRS interval is prolonged (0.08 sec.). Left axis deviation, axis about minus 30°.

A teleorentgenogram taken on admission showed a markedly enlarged globular heart suggestive of congenital heart disease.

An electrocardiogram obtained on entrance to the hospital showed auricular tachycardia with a rate of about 208 per minute, prolonged QRS interval, right bundle branch block, and right axis deviation (Fig. 1*A*).

Since the patient was known to tolerate the drug well, quinidine sulfate were given in doses of 0.1 Gm. (1½ gr.) at four-hour intervals day and night. Five such doses were given without effect, so the dosage was increased to 0.3 Gm. (4½ gr.) every four hours. An electrocardiogram taken the day after admission resembled the previous tracing except that the heart rate was about 200 per minute (Fig. 1*B*). Suddenly, early in the morning of the third hospital day, after a total of 1.9 Gm. of quinidine sulfate (28½ gr.) had been given, the child became markedly dyspneic and cyanotic. Oxygen was given by the nurse, and within fifteen minutes a regular apical rate of 120 per minute was noted by the physician on his arrival. An electrocardiogram taken after this incident showed sinus rhythm with a heart rate of 125 per minute, prolongation of the P-R and QRS intervals, right bundle branch block, and right axis deviation (Fig. 1*C*).

After normal rhythm was established, the heart rate fluctuated between 96 and 140 per minute. With this slowing of the heart rate, a loud rough systolic murmur was audible over the precordium with maximum intensity at the third left interspace. No thrill was palpated. The cyanosis and dyspnea were no longer apparent, and the liver was no longer palpable. Following restoration of normal sinus rhythm, quinidine sulfate was administered in doses of 0.2 Gm. (3 gr.) every four hours for thirteen doses and subsequently 0.1 Gm. (1½ gr.) was given three times a day. An electrocardiogram taken before discharge showed sinus rhythm with a rate of about 100 per minute. The bundle branch block had disappeared, the axis deviation had shifted from right to left, and the P-R and QRS intervals were still prolonged. On March 16, 1941, the patient was discharged with a maintenance dose of 0.065 Gm. (1 gr.) of quinidine sulfate three times a day. The final diagnosis was congenital heart disease with probable patent interventricular septum, paroxysmal auricular tachycardia, and transient right bundle branch block.

On April 11, 1941, thirty-one days after discharge, the child was readmitted in another episode of tachycardia. No precipitating factor was apparent. The patient had been taking 0.065 Gm. (1 gr.) of quinidine sulfate three times a day since leaving the hospital. He was moderately dyspneic and cyanotic. The findings on general physical examination were the same as on the previous admission except that the heart rate was 198 per minute. An electrocardiogram taken the day of hospitalization showed an auricular tachycardia of 198 per minute and a right bundle branch block.

Carotid sinus and ocular pressure were unsuccessful. Holding the child upside down failed to produce any change in the tachycardia. Mecholyl in doses of 5 mg. (1/13 gr.), 5 mg. (1/13 gr.) and 7 mg. (1/9 gr.) was administered subcutaneously at thirty-minute intervals without effect. During the course of this treatment, the carotid sinus and the site of the injections were massaged in an attempt to enhance the effect of the mecholyl, but the tachycardia persisted. Quinidine sulfate was then given in doses of 0.2 Gm. (3 gr.) every four hours. At 8 P.M. on the day after admission the rate was found to have dropped to 128 per minute. This occurred after a total of 1.3 Gm. of quinidine sulfate (19½ gr.) had been given. An electrocardiogram at this time showed normal sinus rhythm with right bundle branch block and prolongation of the auriculoventricular conduction time. Following restoration of sinus rhythm, quinidine sulfate was administered in doses of 0.1 Gm. (1½ gr.) every six hours. At this time the child developed an upper respiratory infection with a temperature of 102.6° F. The heart rate rose to 160 per minute, and the diagnosis of sinus tachycardia was confirmed by electrocardiogram. The heart rate remained accelerated for two

days until the infection subsided, after which it varied between 80 and 100 per minute. No evidence of decompensation was observed during the period of the acute infection. The patient was sent home on a daily maintenance dose of 0.1 Gm. (1½ gr.) of quinidine sulfate.

INCIDENCE

An effort to determine the number of verified cases of paroxysmal auricular tachycardia in infants and children is difficult, because a great many of the cases of rapid heart action in this age group are referred to simply as paroxysmal tachycardia. This is a term which includes paroxysmal auricular tachycardia, paroxysmal nodal tachycardia, paroxysmal ventricular tachycardia, and paroxysmal auricular flutter. In many of the reported cases there is no differentiation between these arrythmias, so that a reliable statistical study of this condition is impossible. About seventy cases of paroxysmal tachycardia in patients up to 15 years of age are recorded in the American and English literature.[1, 2] The majority of these occurred in children rather than in infants. Tarran and Jennings, in 1937, collected fifteen cases of definite paroxysmal auricular tachycardia in infants and children.[2] Since then, four more cases have been reported in the American and English literature.[3, 4, 5]

Johnson[6] believes that this condition is more frequent than is realized, because the tachycardia may easily be overlooked as a result of the mildness of symptoms in young individuals. Furthermore, many adults state that they have had paroxysmal auricular tachycardia intermittently since childhood.

TREATMENT

Although some spontaneous recoveries have been reported in children, energetic treatment of this condition seems necessary because of the danger of cardiac failure with death.[7] Infants may not tolerate a prolonged arrythmia as well as an adult, and several fatal cases of paroxysmal tachycardia of different types have been reported in infants and children.[5] The presence of an organic cardiac lesion renders the prognosis less favorable.

Several well-known measures are employed in attempting to stop paroxysms of auricular tachycardia, but evaluation of therapy as reported in the literature is confusing, since the exact nature of the tachycardia is often not recorded. Also, the dosage of drugs administered and the weight of the patient are frequently omitted in case reports. Little can be found concerning the proper dosage for children. An average adult dose of most drugs can be converted to that suitable for younger patients by employing Young's rule:

$$\frac{\text{Age}}{\text{Age plus } 12} \times \text{Average adult dose}$$

or Clark's rule:

$$\frac{\text{Weight of patient}}{150 \text{ pounds}} \times \text{Average adult dose}$$

1. Vagal Stimulation.—Vagal stimulation by carotid sinus or ocular pressure has been fairly efficacious in children, though the latter is very painful and therefore difficult to perform in a young patient. Holding the breath, compressing the thorax, or swallowing a large bolus of food has sometimes stopped an episode. Production of vomiting by ipecac may be successful. Changes in posture such as laying the baby down with his head lowered or holding the infant suspended by his ankles for a short time have been used.

2. Sedation.—Cases that are precipitated by strong emotional stimulation such as fright or anger may be treated by removal of the disturbing factor from the child's environment, by temporary separation of the child from his exciting surroundings, or by the careful use of sedatives.

3. Sympathetic Paralysis.—Interesting to note is the novacain injection of the stellate ganglion in an effort to interrupt the cardioaccelerator fibers.[8] Cabot[9] reported success in a 4½-year-old boy who had had paroxysmal auricular tachycardia since the age of 2 months in almost monthly cycles. Mecholyl produced no change in rhythm. The left stellate ganglion was injected without response, but injection of the right ganglion resulted in prompt resumption of sinus rhythm. This method was utilized with equally good results on several subsequent occasions.

4. Quinidine Sulfate.—With the possible exception of mecholyl chloride quinidine sulfate is probably the most effective drug used in treating this arrhythmia in adults. Since it is safer than mecholyl and almost as effective, quinidine should logically be used more often in infants and children. It has not been used extensively in this age group because of the pediatrician's lack of experience with the drug.

A few instances of fatal reactions to quinidine have occurred in adults.[7] These probably were due to an unrecognized sensitivity to the medication or to an overdose. Experimental and clinical evidence has been produced, demonstrating that the important factor in toxicity and therapeutic action is the size of the individual dose rather than the total amount given over a period of time.[10, 11] Since the drug is excreted rapidly, cumulative effect need not be feared.

Gold[12] suggests using quinidine sulfate before trying digitalis in paroxysmal auricular tachycardia. The reasons for this are that quinidine sulfate is more often effective than digitalis, and the two drugs may produce toxic arrhythmias if used simultaneously or if quinidine sulfate is given soon after digitalis has been administered. If quinidine sulfate is used initially, enough is excreted in a few hours so that digitalization may be commenced within twenty-four hours without fear of toxic synergism. On the other hand, if digitalis has been given first, at least three to four days should theoretically elapse before quinidine is given to allow time for significant excretion of the digitalis.

By the time that precaution has been observed and therapy with quinidine sulfate started, the infant may be dead due to the uncontrolled tachycardia.

Before treatment is commenced, a test dose should be administered to determine sensitivity to the drug. This dose can be calculated by the use of one of the conversion rules previously mentioned on a basis of 0.1 Gm. (1½ gr.) as the average adult test dose. If the patient tolerates this dose well, active therapy can be instituted. The average single adult dose of quinidine sulfate for use in an acute case is from 0.2 to 0.65 Gm. (3 to 10 gr.) and from 0.1 to 0.2 Gm. (1½ to 3 gr.) for a single maintenance dose. At the Brooklyn Hospital Cardiac Clinic, 4 Gm. (60 gr.) have been administered daily to adults for months with no bad effects. The dose to be given to the child is computed by the use of Clark's or Young's rule and the figures given as the average adult dose. Theoretically this dose would be correct, but it might not be effective, as larger amounts may be required in some cases. So the dose ranges from that suitable for the particular age or weight to an amount slightly in excess of that. The dose should be given every four hours day and night and increased slightly if necessary until either therapeutic or toxic signs appear. The more rapid method of giving five such doses daily at two-hour intervals may be used if preferred. If no result is obtained, the dose may be increased slightly the next day.

Work has been done by Messeloff[10] in regard to use of this drug in children. In an effort to determine tolerance to the drug, as much as 3 Gm. (45 gr.) were given daily to twelve children with rheumatic heart disease whose ages ranged from 8 to 14 years. No toxic effects were noted, and electrocardiographic changes were similar to those seen in adults.

5. Mecholyl Chloride.—A drug which is frequently effective in treating paroxysmal auricular tachycardia is mecholyl chloride. Disagreeable and dangerous side effects result from excessive parasympathetic stimulation and can be abolished by the prompt administration of atropine sulfate by vein. Several fatalities in adults have occurred, but in none of these was any attempt made to control the toxic effect with atropine sulfate.[13] Mecholyl chloride should be given subcutaneously. The intravenous route is not used because of the danger of prolonged asystole. Often a very short period of asystole is observed just prior to restoration of normal sinus rhythm. Ordinarily a dose large enough to induce some slight toxic manifestations is necessary in order to achieve therapeutic success.

Because of the marked reaction in young people, Starr advocates small doses. He believes that the single dose in a patient under 20 years of age should not exceed 10 mg. (⅙ gr.). Starr uses Young's rule and 30 mg. (½ gr.) as the average adult dose in computing the pediatric dose. This is given subcutaneously. If no effect is noted, the

same or a slightly larger dose can be repeated once or twice at thirty-minute intervals.[13] Simultaneous carotid sinus pressure and massage of the site of the injection may enhance the effect of the drug. Gold[14] uses Clark's rule in calculating the dosage. Half of this amount is given at once, and the remaining half is given in fifteen minutes if no toxic or therapeutic effect is observed. Two hours later, after allowing time for excretion of the medication and so presenting cumulative action, a dose 50 per cent greater than the originally calculated amount is given if there has been no effect. A dose approximately twice the original amount is given two hours later provided no toxic or therapeutic effect has been noted. In this manner increasing amounts can be cautiously administered. A syringe containing atropine sulfate for intravenous use should always be at hand.

6. Digitalis.—Digitalis has been useful in some instances where other measures have failed. As mentioned above, its use should follow quinidine sulfate rather than precede it. The patient should be completely digitalized, and a rapid method is usually necessary. The intramuscular route is preferred in infants and young children. It is thought by many that relatively larger amounts of digitalis are required in the younger patient in comparison with those ordinarily used in the older patient. This impression is largely a clinical one and apparently has not been entirely substantiated experimentally.[5] Various schemes for calculating the dose for the child have been proposed, but the following method suggested by Gold seems to be a simple and adequate one. The total amount necessary to digitalize the child fully is approximated by the use of Clark's rule as mentioned before. In calculating this, the average adult digitalizing dose may be regarded as being approximately 1½ Gm. (22½ gr.) of the leaf or 15 c.c. of the tincture, depending, of course, on the potency of the preparation and the reaction of the patient. Half of this computed amount is given orally or intramuscularly, and the other half is divided into two parts to be given at six-hour intervals. If this is not sufficient, the latter dose may be repeated every six hours as is needed, provided the patient is repeatedly observed for clinical or electrocardiographic evidence of toxicity. In this way, an amount larger pound for pound than that given to adults may be administered safely and quickly.[14]

On the other hand, Hubbard[15] believes that digitalis must be administered carefully to infants not in amounts proportionately larger than that used in adults. He uses the Egleston table for all ages, giving 0.1 Gm. (1½ gr. or one digitalis unit) of digifolin intramuscularly per ten pounds in divided doses. In babies this can be accomplished most easily by giving the drug in doses of 0.05 Gm. (¾ gr. or half a digitals unit). Thus the dose administered to the infant is proportionally equal to that given to the adult in contradistinction to the method of Gold previously quoted.

7. Plan of Treatment.—After a study of the literature stimulated by experience with the reported case, the following plan is offered as a rational method of treatment of paroxysmal auricular tachycardia in infants and children.

A. An effort to arrest the tachycardia by vagal stimulation should first be made. This may be accomplished by means of carotid sinus or ocular pressure, postural changes, or induction of emesis by ipecac.

B. Quinidine sulfate should next be tried since this drug is so effective in treating adults. If there is no sensitivity, the drug should be administered at regular intervals day and night. The dose calculated on the basis of the principles mentioned before may be ineffective, so the amount may have to be increased cautiously to one somewhat in excess of the theoretical requirement. Cumulative action is not to be feared as the drug is excreted fairly rapidly.

C. Mecholyl chloride may be reserved until vagal stimulation and quinidine sulfate have been tried. Although this drug is extremely potent, the alarming toxic effects which are sometimes seen can be controlled by the prompt intravenous administration of atropine sulfate in adequate amounts. Small doses must be used as outlined previously, and the drug should be given subcutaneously.

D. Digitalis is thought to be the least effective of these drugs, and consequently it is suggested that it be withheld until quinidine sulfate and mecholyl chloride have failed. It should be used in amounts which are proportionately as large as or larger than the adult digitalizing dose. A rapid method of digitalization is preferable, and the drug should be administered intramuscularly to infants and young children.

E. As a last resort, injection of the stellate ganglion with novacain may be attempted.

This scheme of dosage and administration of the drugs discussed may be applied to the treatment of other cardiac conditions in pediatrics.

SUMMARY AND CONCLUSIONS

1. A case of recurrent paroxysmal auricular tachycardia with transient right bundle branch block and probable interventricular septal defect is reported.

2. The incidence of paroxysmal auricular tachycardia in infants and children is discussed.

3. Paroxysmal auricular tachycardia in infants is a serious condition which may progress to a fatal result and so demands intensive treatment. Proper choice of the drug and adequate dosage are important factors in the success of the therapy.

4. The common methods of treatment are reviewed with emphasis on the method of determining the proper dosage.

5. A plan of treatment for infants and children is outlined.

6. The pediatric dose of some drugs calculated according to Young's or Clark's rule may be ineffective in abolishing this arrythmia. As a result, doses which are larger than those theoretically correct for the individual patient may have to be used. This seems to be true of quinidine sulfate occasionally and of digitalis to a lesser degree.

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THE FAMILIAL EPIDEMIOLOGY OF RHEUMATIC FEVER: GENETIC AND EPIDEMIOLOGIC STUDIES

I. Genetic Studies

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RHEUMATIC fever is a relatively common disease and ranks in importance with tuberculosis as a cause of death in younger age groups. It is a disease of slow evolution, chronic rather than acute, resembling tuberculosis in many respects.[1-4] A study of the epidemiology of rheumatic fever is restricted at the present time by a limited knowledge of the nature of the disease. The etiology of rheumatic fever is obscure, and its incidence in the population is not obtainable. As it is a disease which appears to "run in families," the family unit has offered the best approach for epidemiologic studies.

For more than fifty years the clinical impression that heredity is a significant factor in the concentration of rheumatic fever in certain families has been widespread. This belief was based in large measure on the observed familial incidence of the disease.[5]

There have been several family studies in rheumatic fever. These have considered the relative roles of environment, contagion, and heredity as factors responsible for the high familial incidence of the disease.[6-9] More recent family studies have demonstrated a higher degree of familial association in families with parental rheumatism, and a greater frequency of cases among the progeny of rheumatic families than controls. In some studies of familial association an increased risk for secondary cases in the family has been suggested.[10, 11]

In previous genetic and epidemiologic studies of 112 rheumatic families, it was concluded that *hereditary susceptibility* was the primary factor responsible for the concentration of rheumatic fever in certain families.[8, 12] The present studies comprise a continuation of the epidemiologic and genetic analysis of the same families. The interrelationship of the factors of heredity, age, communicability, and environment is considered. The pattern of intrafamilial spread of rheumatic fever as it refers to *onset* of the disease is described in numerical terms.

It is often stated rather loosely that a disease is hereditary. Familial concentration is commonly observed in contagious, dietary, and parasitic diseases, as well as in hereditary disorders. A disease may not be considered hereditary on the basis of a high familial incidence

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alone; nonhereditary factors must be excluded, and the hypothesis must be substantiated by adequate genetic analysis. Recent progress in the application of genetic theory to human disease makes it possible to investigate the hereditary mechanism which may be operative.

Analysis of the possible role of heredity in the spread of disease is one of the neglected chapters in epidemiology, although it is well recognized that susceptibility of the host is an important factor in the acquisition of disease. The majority of genetic studies has dealt with comparatively rare diseases, and only infrequently have these studies been undertaken in the course of epidemiologic studies.[13-16]

The usual methods of epidemiologic analysis do not permit the evaluation of hereditary factors. In order to study the familial epidemiology of rheumatic fever, analytic procedures, genetic and epidemiologic, were developed which permit the expression of the dynamics of the process. The analytic techniques which have been described in some detail may be found applicable to the integration of genetic and epidemiologic aspects of other comparable problems. A comprehensive consideration of the methods of genetic analysis is included in this presentation since genetic procedures are not readily available in a form to be of use to clinicians for investigation of the role of heredity in human disease.

METHODS OF GENETIC ANALYSIS FOR TESTING THE HYPOTHESIS OF HEREDITY IN HUMAN DISEASE

Requirements of the Data for the Genetic Analysis

Genetic analysis of human family data usually attempts to arrive at a formal description of a given set of data in terms of a Mendelian mechanism of heredity, determined in experimental material. Hereditary studies have been most successful in rare familial diseases where the pathologic picture is characteristic or the condition is congenital.[13, 14, 16] In relatively common conditions the technical methods must be used with caution and the data critically evaluated. It is necessary to consider the particular requirements of data suitable for genetic analysis. With reference to rheumatic fever, diagnostic criteria, age and sex differences, sampling conditions, and other potential variables will be discussed.

Diagnostic Criteria and the Limitations of Historic Data.—In family studies information is infrequently obtained from direct observation of all of the members of the family. More often, complete reliance must be placed upon the statement of the patient or his relatives, or upon records from other sources where different diagnostic criteria may have been followed. The nature of rheumatic fever makes it likely that serious errors will be present in historic records. The manifestations of the disease are often subclinical or mild and pass unnoticed. Furthermore, adults frequently do not recall their childhood experiences.

Since published family studies have usually been based on historic records, it seemed desirable to determine the extent to which reliance might be placed on such information.

There were available eighty-seven families for whom a detailed "family history record" which had been compiled previously could be compared with the results of subsequent follow-up and examination of every member of the household. The subsequent analysis includes a small number of individuals born after the initial history was compiled and a number of children who developed rheumatic fever after the initial family history was obtained.

Comparison of the data obtained on history and after observation is presented in Table I of the Appendix.* Rheumatic fever was reported in only sixteen parents and was found, on examination, in thirty-nine parents. (The proportion of families with one affected parent increased from 18.4 per cent to 44.8 per cent.) This analysis also revealed a deficiency in sibling cases. About 28 per cent of the sibling cases were not identified in the historic records, including a small number that occurred among the thirty-six siblings who either were not recorded in the historic record or were born after it was made.

The necessity for complete medical verification of historic pedigrees in rheumatic fever is apparent from these observations. That this source of error is present in other family studies may be judged from the statement of Irvine-Jones, ". . . it was found that the more complete the examination, the higher the proportion of persons found to be rheumatic."[7]

It is obvious that diagnostic criteria must be adequate and equally applicable to all individuals included in the analysis. The evaluation of diagnostic criteria is primarily a clinical matter. Unrecognized diagnostic errors may lead to erroneous interpretations of the data. While such errors might not be revealed in a general genetic analysis, inconsistencies would appear when the data were subdivided for more detailed study. Furthermore, diagnostic errors are not apt to be distributed throughout the sample at random, and they are more likely to occur in the parents. Inaccuracies in parental diagnosis are significant since the genetic estimate for affected siblings will be incorrect in every family in which the parental diagnosis is in error.

In this sample of eighty-seven families, most of the errors consisted in an understatement of cases per family, as would be expected from the nature of the disease. For example, between one-fourth and one-third of the families would have erroneous genetic estimates for sibling incidence if the information from the historic records alone were used. Instances of the opposite trend were also found (Table I, Appendix).

The Factor of Age.—The age expression of a disease must be considered in applying genetic techniques to clinical data, except, of course, for conditions that are invariably congenital and apparent at birth.

*All Appendix tables will appear in the reprints.

Where the usual age of onset of a disease occurs in the adult or later life, as in diabetes or hypertension, nonaffected individuals must be followed for a period sufficiently extensive to make reasonably certain that they will not develop the respective condition.[17] The mortality due to other causes prior to the critical years of high case incidence must also be considered. In rheumatic fever this problem is simplified by the occurrence of the majority of onsets in childhood. However, when siblings in the family have not reached the maximum age of onset of the disease, the age variable must be taken into account. Deaths from other causes during this period are sufficiently infrequent to render this possible source of error unimportant.

Sex Differences.—Real differences in case incidence between the sexes may be of considerable importance in the interpretation of any genetic analysis. If the *selection* of data is such as to favor one sex among the patients, there will be a disproportionate excess of that sex among the index cases and this may result in an erroneous interpretation of the genetic analysis.

Sampling.—

General Selection of Families: Routine procedures in the accumulation of clinical material are likely to introduce several types of bias. These may include age and sex differences, as well as economic, racial, or environmental sources of bias. These selective factors must be evaluated with great care and appropriately stated. Whenever possible, they should be taken into consideration in the relevant analysis. It must here be emphasized that the methods used to correct sampling errors must not have the effect of masking real differences in the data.

Perhaps the most important sampling bias occurs in the selection of *families* for a genetic study. There is a tendency to include families with a high familial incidence, eliminating others when the case incidence is known or believed to be low. This type of selection governs *precisely those features which are being studied.* It is just as fallacious to conclude that heredity is important in families deliberately selected for high case incidence as it would be to draw the opposite conclusion from families selected because there was never more than one case present. It might be inferred that when part of the material has been selected on the basis of high case incidence, the error could be compensated for by the addition of families known to have a low case incidence. It must be emphasized that such methods of selection cannot be employed in the collection of data for family studies.

The selection of a sample wholly without bias is almost impossible to realize in clinical material. However, there are a number of analytic procedures which make it possible to utilize clinical data with certain types of specified bias.

In investigating the role of heredity in familial association, it is necessary to determine to what extent the presence of rheumatic fever (or other conditions)

in a family (brothers and sisters) is influenced by its presence in the parents or other ancestors or collateral relatives. This is a simplification of the general problem which is not sufficient for the investigation of certain complex problems, such as mutation, but is fully adequate for the purposes of this study. An ideal sample would be one selected without reference to the familial incidence among the members of a kinship.

These conditions would probably be met if the study were made on individuals born within a specified and short-time interval (that is, six months) without reference to the possible presence of the condition among them or their kinfolk. It is apparent that it is neither feasible nor practical to obtain an ideal sample by this method. For all except the most common conditions it would yield only a small amount of significant data.

The Index Case: The study of familial incidence usually starts from a known case in a patient in attendance at a clinic (the index case). When the index case is a *sibling,* the influence of heredity is measured by a comparison of the incidence in sibships when one parent is affected, both parents are affected, and neither parent is affected. When the index case is a *parent,* the comparison of sibling incidence is made between the groups in which the unspecified parent is affected and in which the unspecified parent is unaffected.

For the types of information usually available about *siblings,* families with the following types of bias may yield information concerning heredity:

1. Sibships with one known affected member (and no information regarding the possible presence of other affected members).

2. Sibships with two known affected members (and no other information). (These can be extended for higher known case incidence, but the analysis is different for each specified condition.) The first type of bias usually occurs in data collected from a children's clinic.

Whenever there is a partial selection for multiple case incidence, the errors will be minimized by making an appropriate correction for two (or if necessary three or more) known cases. This would, however, require the exclusion of sibships with only one affected member, regardless of size.

When the information concerns the *parents,* other conditions may be specified; for example, occurrence in one or both parents or the occurrence of the condition in one or more grandparents or other specified relatives (except children). It must be noted that summations are permissible only for those families in which the same or equivalent specifications are provided. In rheumatic fever, when selection of data is made from an adult cardiac clinic, one affected parent is usually specified.

When the family data are recorded irrespective of the parental diagnosis, the relative frequency of the condition in the children is of little consequence. The significant measure of heredity in this type of analysis is association with parental incidence (as affected members or genetic "carriers"). Therefore, differences between mating combinations must be maintained for effective analysis. In several published studies,

selection was based on a minimum of two cases per family, without differentiation between sibling and parent cases. A parent case has a far greater genetic significance than a sibling case, since it affects the familial incidence (except for simple dominant inheritance). In a mixed sample of parent and sibling selection, the evaluation of heredity cannot properly be made.

Other Potential Variables.—Important nongenetic factors relative to the disease or condition must be considered with reference to the genetic analysis. For example, in rheumatic fever differences in economic status may be of considerable importance. In making the genetic analysis, the data must first be analyzed separately for each economic group represented. If no difference is found, the variable may be disregarded and the data need not be subdivided subsequently with respect to this variable. Sex differences and other potential variables may be treated similarly.

TABLE I

AGE DISTRIBUTION OF CHILDREN*

DIAGNOSIS OF PARENTS	NUMBER OF FAMILIES‡	AGE (YR.)				TOTAL
		0 TO 4	5 TO 9	10 TO 15	16+	
Both negative†	59	10	33	84	124	251
One parent rheumatic	50	10	32	70	93	205
Both parents rheumatic	4	0	2	6	7	15
All families (total)	113	20	67	160	224	471

*Thirty-five children who died before their second birthday have been omitted from this and succeeding tables.

†A negative parent is non-rheumatic.

‡There are included four second marriages with step-siblings, noted under the phenotype of their proper parents.

Description of the Data Used for the Genetic Analysis of Rheumatic Fever

One hundred and nine households comprising 113 genetic families (including remarriages) were originally selected from the children's cardiac clinic for another investigation.[18] Every household included at least one rheumatic child. At the time of final tabulation of the data shown in Table I (Jan. 1, 1939), 96 per cent of the siblings in 113 families used for pedigree analysis had reached the age span of maximum incidence, and 82 per cent had passed it. The period of observation ranged from six to twenty years, and all but twenty families were observed for more than nine years. The distribution of cases in each family is shown in Table II of the Appendix.

The relative frequency of rheumatic fever in the families did not influence the selection. The sole criteria were (1) residential propinquity for convenience in social service follow-up and (2) willingness to cooperate in a series of bacteriologic studies which necessitated weekly visits to the home. Three of the original 112 families[12] were excluded from these studies because the information available was not adequate for detailed analysis.

Many of the families were under observation in the general clinic preceding the onset of the first sibling case. In most instances all members of the families were examined, and a continuous medical record was kept of each individual. The detailed diagnostic criteria as well as age, sex, and other general sampling characteristics have been presented elsewhere.[12] In most respects, the negative and positive parent families are quite similar. In Table I the age distribution of the children according to parental diagnosis is presented. Table II of the Appendix gives the distribution of affected siblings with respect to family size.

In all essential respects these data fulfill the requirements for genetic analysis previously presented. It is to be noted that selection was based on the presence of at least one sibling case per family.

Review of Genetic Principles and Methods

Evidence that heredity is an essential element in the familial expression of any disease or condition is available if the distribution of cases is consistent with the general laws of inheritance and if agreement with a specific hereditary mechanism is obtained. It may be noted as a familial concentration within a sibship or in succeeding or contemporary generations of a kinship. Other possible factors responsible for the familial association observed must be evaluated.

Twin Studies.—The incidence of a disease or condition among twins is frequently used to establish the possible role of genetic factors. Such studies have given results consistent with the hypothesis that hereditary factors are important in rheumatic fever.

Twins occur with relative frequency and may be studied more easily than ordinary siblings. The differences between fraternal and identical twins are, at least in part, genetic. Differences between fraternal twins are similar to differences between other siblings, while the differences between identical twins are never regarded as genetic in origin. It would be expected that traits in which heredity plays a prominent part would not occur as frequently in both members of a pair of fraternal twins as in an otherwise comparable pair of identical twins. A number of twin studies have been published in rheumatic fever.[7, 12, 19] In our records there were seven pairs of twins, of which two pairs were identical. The two pairs of identical twins were alike in having rheumatic fever. Among the fraternal twins, two pairs were similar, i.e., either both were rheumatic or nonrheumatic, and three pairs were dissimilar. In the large series of Kaufman and Scheerer a similar trend was noted.[19]

There are several precautions which should be considered in the evaluation of data of twins. Twins share their environment in a greater measure than ordinary siblings. The diagnosis of zygosity is often based on superficial resemblance and may be prejudiced by the presence or absence of the trait under investigation. There is the additional assumption in twin studies that other possible influential factors are alike for both. While this is likely to be true in any pair of twins, it may be otherwise in a different pair of twins. With these reservations, however, twin studies are valuable as corroborative evidence in hereditary studies.

Consanguinity.—Consanguinity must be taken into consideration in determining the hereditary mechanism when the maximum prevalence of the condition in the general population is less than 0.1 per cent. In rare recessive traits a high family incidence may be due to consanguinity. Since rheumatic fever is far more prevalent than 0.1 per cent in the general population, cousin marriages were not used to test the interpretation of recessive inheritance.

Pedigree Analysis.—In the medical literature the simplest type of analysis is a comparison of the ratios of affected individuals to total number of individuals for various degrees of kinship to the index case. Since several generations and degrees of kinship are usually combined in these computations, the interpretation of heredity cannot have specific connotation. This type of analysis has little value except as corroborative evidence in certain rare circumstances.[20]

A more satisfactory simple measure of familial association is a comparison of the ratios of affected siblings to total number of siblings for several types of matings.

TABLE II

FAMILIAL INCIDENCE OF RHEUMATIC FEVER IN 113* FAMILIES

	NUMBER OF SIBLINGS PER FAMILY	NUMBER OF FAMILIES*	TOTAL SIBLINGS	NUMBER RHEUMATIC	PER CENT RHEUMATIC	AVERAGE NUMBER OF CASES PER FAMILY
A. Both parents negative	1 to 3	24	61	32	52	1.3
	4 to 6	27	128	51	40	1.9
	7 to 9	8	62	18	29	2.2
Total		59	251	101	40	1.7
B. One parent rheumatic	1 to 3	24	53	37	70	1.5
	4 to 6	18	87	48	55	2.7
	7 to 9	8	65	32	49	4.0
Total		50	205	117	57	2.4
C. Both parents rheumatic						
Total		4	15	14	93	3.5

*Four families in which both parents were rheumatic were excluded from subsequent analyses.

For example, from the summary of family incidence in our data (Table II), it is apparent that the average number of cases of rheumatic fever is greater in those families in which the parents had suffered from the same disease in childhood, in comparison with families in which the disease was not known to have occurred in the parents. In the larger families, with seven to nine siblings, the bias due to selection of families from a clinic population (insuring at least one affected member per family) is minimized. In this group there are nearly twice as many affected members per family where one parent is rheumatic. The sibling case rates in the larger families approach 50 per cent and 25 per cent, respectively. Among the four families in which both parents were

rheumatic, fourteen of the fifteen siblings were rheumatic. The only nonrheumatic child had not yet reached puberty.

These observations and the numerical values presented in Table II suggest a single recessive gene mechanism of inheritance.

For many years following the demonstration of Mendelian inheritance in man (brachyphalangy by Farabee in 1905[21]), family trees (pedigrees) were used to chart the descent of disease or defect in human families. The occurrence of a trait in one-half the progeny in common with a parent, or in a quarter of the progeny when both parents were free of it, was regarded as conclusive proof of dominant and recessive inheritance, respectively. Appropriate ratios for sex linkage and more complex hereditary mechanisms were also used.

In Table III are presented estimates of affected progeny for certain common matings.

TABLE III

ESTIMATE OF AFFECTED PROGENY FOR CERTAIN COMMON MATINGS

HEREDITARY MECHANISM	BOTH PARENTS NEGATIVE	ONE PARENT POSITIVE	BOTH PARENTS POSITIVE
Dominant	None	*One-half*	All
Recessive	*One-quarter**	One-half	All
Sex-linked	*Sons: One-half* *Daughters: None*	Father + : None Mother + : All sons no daughters	All

*The mating type *(italicized)* is the one most commonly encountered clinically for each mechanism of inheritance. Negative parents are considered carriers.

For example, in the case of brachydactyly, the usual mating encountered is positive by negative. When the ratio of affected to total number of siblings is made over the entire range of family sizes, the computed genetic radio is ½ (0.50). Matings of an unaffected relative with any normal individual would yield no cases among the offspring. This analysis may be interpreted as indicative of dominant inheritance. From an examination of the pedigree it is often possible to make certain additional qualitative observations which would tend to confirm or question the interpretation inferred from these computations.

Pedigree analysis may be used very effectively under certain circumstances.[13] For example, when a relatively uncommon, genetically suspect condition is passed directly from a parent to many of his children in several generations (as occurs in many congenital ocular and skeletal defects), the mechanism of simple dominant inheritance may be deduced with relative ease. Or, in Y-chromosome, and often X- (or sex) chromosome inheritance illustrated by partial color blindness, a simple pedigree analysis may be adequate. In certain relatively rare diseases such as alkaptonuria or infantile amaurotic idiocy, the pattern of genetic transmission may be deduced by these simple methods.

Occasionally a single extensive family tree of a clearly defined clinical entity may be sufficient to establish the mechanism of heredity. This is illustrated by the pedigree of congenital stationary night blindness recorded by Nettleship in 1907,[22] covering nine generations with a total population of 2,116 persons.

The evaluation of human pedigrees in the early studies of pellagra, tuberculosis, and schizophrenia, among many others, by the use of the simple formulas of Mendelism has led to a recognition of the limitations of this procedure.

Failure to recognize these limitations has led to claims for the hereditary nature of various diseases on the basis of evidence which is inadequate. It has been shown that the frequently quoted simple recessive inheritance of schizophrenia was based on this misconception. While very few affected members were found additional to the index case in nearly 700 families, the ratio of affected to total number of children was nearly 25 per cent (¼) due to the fortuitous circumstance that the average family size was approximately four.[23, 24]

In using the simple genetic ratios, the assumption is made that the clinical data are representative of the matings for each type in the general population. This is rarely attained in ordinary clinical material due to bias in the selection of the families for study. For in view of the small size of human families in comparison to experimental material, certain families should occur in the general population without any positive siblings, by chance alone. Such families would not be represented in samples drawn from a clinic population, which almost invariably includes at least one case per family.

In recessive matings, the unaffected parents are assumed to be heterozygous (the homozygous negative parents are not represented in a clinic sample since their offspring would include no affected members). In the sex-linked mating of two negative parents, the negative mother is presumably heterozygous. In most instances there is no independent evidence that the negative parents are heterozygous. This type of analysis has further limitations in conditions where nongenetic components are operative, as for example, the presence of a specific etiologic agent, or characteristic environmental conditions, or age and sex specific differences with regard to the disease in question. These restrictions limit the number of diseases which may be satisfactorily investigated by simple summation from pedigree records.

Modified Methods for Genetic Analysis

The Proband and Sibling Methods.—Weinberg has proposed two corrections for the analysis of human family data.[25] In the *proband* method, the index case is excluded, and the ratio is computed from the siblings of the index case. This eliminates the bias due to selection of at least one case per family but excludes a portion of the data. The method is accurate only in instances of complete ascertainment of cases,[26] a condition which is not often met in clinical data. It is not adequate for predicting total case incidence in a family sample.

In the *sibling* method every sibship is counted as many times as there are cases, each case being in turn regarded as the index case. This procedure may be inaccurate if diagnostic errors are present, since every error is multiplied as many times as there are siblings.

The Lenz-Hogben Formula.—The use of simple Mendelian ratios for genetic analysis may be expressed in terms of varying family size and

total affected siblings by the following general formula: $R = \sum_{s=1}^{s=c} Sn_s p$. This formula assumes that mating occurs at random, and that the sample has no selective bias. In such a sample, theoretical genetic ratios may be substituted for p (that is, ¼ or ½) and an expected number of cases, R, may be computed. The extent of agreement between expected and actual cases is a measure of the suitability of the genetic hypothesis.

The usual sample of clinical data, however, is biased by the inclusion of at least one case per family. Lenz has modified this formula to correct for the families of the same genotype in the general population who have no cases among their offspring and would not be represented in a clinic sample. Hogben has derived an expression for the standard error in the Lenz equation, making it possible to test the adequacy of specific genetic interpretations by a comparison of the observed and expected case frequencies. The Lenz-Hogben formula has been applied to the analysis of more than thirty human hereditary traits.[27-29]

Haldane,[30] and independently Bernstein,[31] by a method of inverse probability compute the most likely value for the occurrence of the condition in the general population from the observed familial incidence in the sample. This is compared with the requirements of single and multiple gene hypotheses. Proofs of these formulas have been presented in a series of papers by Hogben[27, 32] and Haldane.[27, 29, 30] Haldane has also shown the relation between the two formulas. (Other methods based on the analysis of gene frequency have also been developed, but these were not for the most part suitable for the available data.)

The Lenz-Hogben formula for predicting the total number of cases in a series of families is:

$$R = \sum_{s=1}^{s=c} \frac{Sn_s p \pm \sqrt{n_s k_s}}{1 - q^s}$$

R = Total cases expected
c = Maximum family size
s = Family size
p = Frequency of affected offspring, i.e., the genetic ratio
q = Frequency of normal offspring, when $q + p = 1$
n_s = Number of families of s size
Sn_s = Total siblings in families of s size

$$k_s = \frac{s(1-q)}{(1-q^s)^2} \times [sq^s(q-1) + q(1-q^s)]$$

where all the terms are defined above, and the standard error is given by $\sqrt{n_s k_s}$. Since the values of s, p, and q, as well as k_s are constants, it is possible to derive a series of factors for use in genetic prediction which simplify the numerical computations. Factors for other mechanisms than recessive inheritance may be derived in a similar manner, by using appropriate values for p and q. Table IV gives the computed values of $sp/1-q^s$ and of k_s which may be used as genetic factors in computing expected cases for any recessive trait.

The analytic justification for the Lenz correction of the original formula is as follows:

It has been proved experimentally that the frequency distribution of affected members and unaffected members exhibiting an hereditary disease may be described by the binomial expansion of $(q + p)^s$ where q is the frequency of normal offspring, p is the frequency of affected offspring, s is family size, and $(q + p) = 1$. For example, in a group of eight three-membered experimental families, the relationship would be expressed as follows:

$$(q + p)^3 = q^3 + 3q^2p + 3qp^2 + p^3$$

The q^3 term corresponds to the proportion (one-eighth) of such families which will have no cases (p^0); the $3q^2p$ term corresponds to the proportion (three-eighths) of families which will have one case (p^1); the $3qp^2$ term corresponds to the proportion (three-eighths) of the families which will have two cases (p^2); the term p^3 corresponds to the proportion (one-eighth) of families who will have three cases. It then follows that for every family of the given genotype in the total population having no cases, there will be three families having one case, three families having two cases, and one family having three cases (coefficients of the binomial expansion to the third power: 1, 3, 3, 1).

It has been inferred that this general rule of genetic distribution occurs in man as well and may be used to obtain estimates of case frequency when applied to suitable human data. Human families in the general population corresponding to the q^3 term are not recognized. They are therefore not represented since their offspring include no cases which would identify the parents as carriers. It is therefore necessary to modify the expansion of $(q + p)^s$ in order to make it applicable to data biased by the presence of at least one case (or a greater number of cases if such a situation arises). This may be done algebraically by eliminating the q^3 term without destroying the interrelationship between the other terms:

$$\begin{aligned} (q + p)^s &= 1 \\ (q + p)^s - q^s &= 1 - q^s \\ \frac{(q + p)^s}{1 - q^s} &= \frac{1}{1 - q^s} \end{aligned}$$

It is then apparent that the general formula $R = \sum_{s=1}^{s=c} Sn_sp$ which gives an estimate of the frequency of the condition in the general population may be modified by dividing by the term $1 - q^s$ and will thus include a statement of the proportion of case frequencies in a group containing at least one case per family. It is important to emphasize that the application of the simple genetic ratios of one-quarter or one-half is not analytically justified in a sample biased by the inclusion of at least one case per family, inasmuch as the theoretic ratio of cases in a biased sample is considerably higher, particularly in the smaller family sizes.

Genetic Analyses in Rheumatic Fever

Procedure for Predicting Cases Using the Lenz-Hogben Formula.—The application of the Lenz-Hogben formula for predicting the total number of cases in a series of families is simplified by the use of the genetic factors described above (Table IV).

To compute the expected number of cases in a group of rheumatic families of given size, the appropriate factor (Table IV) is multiplied by the number of families of that size (n_s). The standard error (σ^2) is obtained by multiplying the appropriate value of k_s by the number of families. For example, the expected number of cases in the two-membered families in our data is obtained by multiplying the factor 1.143 by 9, the number of two-membered families. Its variance (σ^2) is obtained by multiplying 9 by 0.122. This procedure is followed for every group of families arranged according to family size and parental diagnosis.

TABLE IV

FACTORS DERIVED FROM THE LENZ-HOGBEN FORMULA FOR USE IN GENETIC PREDICTION

FAMILY SIZE	BOTH PARENTS NEGATIVE ($q = 0.75$ $p = 0.25$) $sp/1 - q^s$	k_s	ONE PARENT POSITIVE ($q = p = 0.50$) $sp/1 - q^s$	k_s
1	1.000	0.000	1.000	0.000
2	1.143	0.122	1.333	0.222
3	1.297	0.263	1.714	0.490
4	1.463	0.420	2.133	0.782
5	1.639	0.592	2.581	1.082
6	1.825	0.776	3.048	1.379
7	2.020	0.970	3.528	1.667
8	2.222	1.172	4.016	1.945
9	2.433	1.380	4.509	2.215
10	2.649	1.592	5.005	2.478
11	2.871	1.805	5.503	2.737
12	3.098	2.020	6.001	2.992
13	3.329	2.234	6.501	3.245
14	3.564	2.446	7.000	3.497
15	3.801	2.658	7.500	3.748
16	4.041	2.867	8.000	3.999
17	4.282	3.074	8.500	4.250
18	4.526	3.279	9.000	4.450
19	4.770	3.481	9.500	4.750
20	5.016	3.682	10.000	5.000

Values of k_s are computed from Hogben's formula.

Values of $s/1-q^s$ have been computed from Lenz-Hogben formula for the several values of p and q in our data; ($sp/1 - q^s$ may be computed for each family size and applied as a genetic factor for prediction).

Results of Genetic Analysis.—The genetic analysis of 109 rheumatic families is presented in Table V. Among fifty families in which one parent was rheumatic ($p = 0.50$), the observed frequency of 117 rheumatic children is not significantly different from the expected value of 111.9 $\pm$6.43, the deviation being 0.79σ. In the families with two non-rheumatic parents, 101 cases among 251 offspring were observed in com-

parison with 90.0 ± 5.35 cases expected ($p = 0.25$). The deviation is on the borderline of significance, 2.06σ.

The use of the Lenz-Hogben formula to estimate the number of cases which should be observed is illustrated by this analysis. The agreement obtained, assuming theoretic frequencies of 0.25 and 0.50, constitutes presumptive evidence in favor of a single recessive gene mechanism in rheumatic fever.

TABLE V

CASES EXPECTED AND OBSERVED USING THE LENZ-HOGBEN FORMULA

FAMILY SIZE	NUMBER OF FAMILIES	TOTAL CHILDREN	RHEUMATIC CHILDREN		DIFFERENCE	σ^2	DIFFERENCE/σ
			OBSERVED	EXPECTED			
A. Both Parents Negative							
1	1	1	1	1.0	0.0	0.00	0.00
2	9	18	11	10.3	0.7	1.10	0.67
3	14	42	20	18.2	1.8	3.68	0.94
4	12	48	19	17.6	1.4	5.04	0.62
5	10	50	20	16.4	3.6	5.92	1.48
6	5	30	12	9.1	2.9	3.88	1.47
7	3	21	9	6.1	2.9	2.91	1.70
8	4	32	8	8.9	−0.9	4.69	0.41
9	1	9	1	2.4	−1.4	1.38	1.19
Total	59	251	101	90.0	11.0	28.60	2.06
B. One Parent Positive							
1	6	6	6	6.0	0.0	0.00	0.00
2	7	14	10	9.3	0.7	1.55	0.56
3	11	33	21	18.9	2.1	5.39	0.90
4	9	36	22	19.2	2.8	7.04	1.01
5	3	15	9	7.7	1.3	3.25	0.72
6	6	36	17	18.3	−1.3	8.27	0.45
7	2	14	7	7.0	0.0	3.33	0.00
8	3	24	9	12.0	−3.0	5.84	1.24
9	3	27	16	13.5	2.5	6.64	0.97
Total	50	205	117	111.9	5.1	41.31	0.79

Further Analyses in Support of the Single Autosomal Recessive Gene Hypothesis in Rheumatic Fever

The Analysis of Other Data for Corroborative Evidence.—Once a genetic hypothesis has been established for a given set of human data, the best type of corroborative evidence may be obtained if the hypothesis is found to give good agreement when another set of comparable data is analyzed.

The opportunity for making a preliminary analysis of another set of rheumatic fever data was available through the courtesy of Dr. John R. Paul. The data suitable for genetic analysis consisted of a series of fifty-eight families in which both parents were negative, selected with a bias of at least one case per family. The information given at the time of analysis was the parental diagnosis and the number of families of varying sizes.

The Lenz formula was used to predict the number of expected cases. Subsequently, the actual number of cases in each family was given to us for comparison. It was found that for fifty-eight negative parent families ranging in size from one to twelve, the total cases expected was 106.7 and the total number actually observed was ninety. The standard error was 2.5σ. There was a consistent deficiency of cases in every family size, although in no instance was this deficiency significant. It may be concluded from this analysis that these data furnish confirmatory evidence in support of the mechanism of single recessive inheritance in rheumatic fever. It is to be emphasized, however, that this analysis alone could not be offered as proof of the hypothesis unless more extensive analysis of the data would give a satisfactory explanation for the deficiency of cases. This would consist in a review of the requirements of the data for genetic analysis as well as further analytic procedures.

Internal Consistency of the Data.—Further genetic analyses were made on the data which provide corroborative evidence in favor of the genetic mechanism postulated. These include tests for the internal consistency of the data and the application of other genetic formulas.

Multiple Case Predictions.—It has been shown that the correction for the sampling condition of one sibling case per family (that is, the Lenz formula) is made from the expansion of the binomial. By an extension of this method, factors may be derived which yield estimates for samples with the bias of selection for two or more cases (See Appendix, Tables IIIA and IIIB). These factors may be used for data selected with a bias of at least one case per family by treating in turn those families in the group having two or more, three or more, etc. cases.

Table IV of the Appendix gives the factors derived for families ranging in size from one to ten siblings and having at least one, two, three, four and five cases.

When these factors were applied to our data, good agreement was obtained (Appendix, Table V). This extension of the Lenz formula for application to subdivided groups of the data demonstrates the internal consistency of our data with the hypothesis of single recessive inheritance.

The Application of Other Genetic Formulas to the Data.—Formulas have been derived by Haldane and Bernstein which may be used to obtain another type of information from data biased by the selection of at least one case per family.

In Haldane's formula, the most probable value of q (and of p) is estimated in the data under analysis. If the value of q derived from the data does not differ significantly from the theoretic value, it may be assumed that the data follow the postulate of a particular genetic mechanism (that is, if q does not differ significantly from 0.75 in a sample of negative parent families, other conditions being consistent, the mechanism is that of a single recessive gene).

Table VI of the Appendix gives the estimated number of expected cases, using the Haldane formula, for several values of q. The best agreement is obtained when $q = 0.681$ and p therefore is 0.319. The standard deviation for the most probable value of p is 0.319 ± 0.041. The deviation from a single recessive gene hypothesis (0.25) is 0.069, which is not statistically significant (1.68σ). (The value of 1.68σ may be interpreted to mean that there would be 9 chances in 100 trials of obtaining a deviation as great as or greater than that observed.) The results of similar computations on the mating of affected by unaffected parents are also consistent with the interpretation of single recessive inheritance.

The hypothesis of two complementary recessive genes ($q = 0.875$, $p = 0.125$) may be excluded inasmuch as the deviation of 0.194 is more than five times the standard error of the expected value (5.42 σ). This deviation is so great that the odds are several million to one against the chance occurrence of a deviation of that size. The interpretation of more than two recessive genes would necessitate the use of even larger values of q and consequently need not be considered further.

Bernstein[31] has given a variation of this formula which permits an independent estimate of the value of q (and p) for each family size (Appendix, Table VII).

The results of the analysis of our data, using the Haldane and the Bernstein formulas, are equally consistent with the interpretation of single recessive inheritance. The breakdown of the data by family size, using the Bernstein formula, made it possible to determine whether some family sizes were selected with more or less bias than others with regard to case incidence. No significant difference was observed.

Neither the Haldane nor the Bernstein formula as used above permits a direct computation of "expected cases." For it is apparent that if the actual number of cases observed is used to derive an absolute value for the genetic ratios, these ratios cannot in turn be used to predict an expected number of cases. These formulas provide a means for expressing the prevalence of cases in a series of families in terms of a genetic distribution. From this it is possible to infer the type of genetic mechanism which is operating.

Comparison of the Actual and Theoretic Binomial Distributions.—The foregoing formulas which are used to test the consistency of the data with a specific genetic mechanism are dependent upon the degree to which the binomial expansion of $(q + p)^s$ describes the distribution of affected families. The data may therefore be tested for consistency with the genetic mechanism by a direct comparison of the distribution of affected families with the theoretic distribution derived from the binomial expansion (Appendix, Table VIII, A and B). Adequate agreement was obtained in most instances in all family sizes in both mating combinations (Appendix, Table IX).

If systematic deviations from the binomial expansion are observed throughout the data, they may be the result of sampling criteria or diagnostic errors. Occasional deviations such as those found in our data may be traced and attributed to specific families. This use of the binomial expansion provides a simple method for determining whether or not a group of data is internally consistent with a postulated genetic hypothesis.

Evaluation of Numerical Differences Between Expectation and Observation

In the preceding discussion supportive evidence has been given to the hypothesis of simple recessive inheritance in rheumatic fever. The agreement between observed and computed values for each family size in the various tabulations and the agreement with the binomial distribution in every family size would not be expected if the interpretation of recessive inheritance were in error. In attempting to evaluate the numerical differences between expectation and observation, several possible sources of error were investigated; namely, selective factors and parental diagnosis.

It will be recalled (Table V) that there was an excess of observed cases in the negative parent families which was on the borderline of significance (2.06 σ), although in no instance did the line-by-line difference exceed an error of 2 σ.

Selective Factors.—In accounting for the difference between expected and observed case incidence, it first seemed desirable to determine whether any inadvertent

selective factors were operative during the period of years when these families first came under observation in the clinic. The data were divided into arbitrary time intervals by treating separately the families that came under observation before 1925, from 1925 to 1931, and from 1932 to 1939.

The analyses for each time interval, using the Lenz formula, are presented in the Appendix, Table X. In most instances the errors (difference: sigma) were not statistically significant. This indicated that the sample was homogeneous.

However, there were a few significant differences. Satisfactory agreement was found in two of the three groups of negative parent families. The excess of observed cases in the group selected between 1925 and 1931 where the difference was greater than 2σ appeared to be due to the excess in the three families with six and seven siblings.

In the positive parent families an excess occurred in the group of families selected before 1925 and may be attributed to the group of five families with four siblings. However, it is to be noted that this excess was not revealed in the total values in Table V, as it was compensated for by the close agreement in the remaining groups. The importance of inspection for line-by-line agreement is here illustrated.

Parental Diagnosis.—It was considered that possible inaccuracies in parental diagnosis might account for the excess of affected members in the three negative parent families specified above. To investigate this possibility, forty-six families were selected for separate tabulation. These included only those families in which possible inaccuracies in the diagnosis of both parents could be eliminated. The analysis of these families using the Lenz formula is shown in the Appendix, Table XI.

Close agreement (standard error less than 1σ) between expected and observed cases was obtained throughout the analysis of this selected group of families. From this analysis it may be inferred that the difference in the negative parent families (about 2.1σ) should be attributed to the inaccurate classification of a few parents.

It will be recalled that errors in the diagnosis of a parent are genetically far more significant than similar errors in a sibling, although the latter would tend to produce a deficiency of affected members. Occasionally, when such errors occur at random, they may compensate for each other and will not be apparent in the final analysis. However, in rheumatic fever, diagnostic errors are more likely to be due to the lack of recognition of a rheumatic parent, and to a lesser extent, a rheumatic sibling.

In our data rigid criteria were followed for an affirmative parental diagnosis, so that a greater proportion of errors would be expected in the group of parents classified as nonrheumatic. One or two parents, incorrectly classified, might well be sufficient to account for the entire excess observed in the sibling classes. It has already been shown in Table V that the differences in the negative parent families are largest for the family sizes of five, six, and seven. In the Appendix, Table X the only family sizes with significant differences occur in the group which first came to the clinic between 1925 and 1931, in the family sizes of six (including two families) and of seven (including one family). Review of the original records of these three families revealed that in two reliance was placed on historic records in considering one of the parents in each of these families nonrheumatic. In one instance death was due to hypertensive heart disease, in another tuberculosis. In the third family, there was evidence of simple mitral insufficiency of unknown etiology in one parent. It is significant that the most important differences between the binomial distribution and the observed family distribution is due to these three families (Appendix, Table IX). Elimination of these three families from the genetic analysis of the negative parent families results in close total agreement (84.3 cases expected and 88 cases observed, 1.5σ).

It was concluded that the excess of cases observed in the negative parent families could be attributed to the possible inaccurate diagnosis of the three parents who have been identified in the preceding analysis.

Elimination of Alternative Mechanisms of Heredity.—Although the various analyses presented in the preceding discussion are indicative of simple recessive inheritance, it was necessary to exclude other possible hereditary mechanisms. Simple dominance, sex linkage, complementary recessive (two or more genes), and complementary dominant inheritance were considered and excluded.

Simple Dominance.—Any hereditary character which is transmitted as a simple dominant should be expressed in every generation in approximately one-half the progeny of a family in which the trait is found. In our data the absence of the disease among the parents of about one-half of the families is inconsistent with this hypothesis. Irregular dominance, that is, a dominant mechanism which is incompletely expressed or is not consistently apparent, was rendered unlikely in our data by the occurrence of rheumatic fever in fourteen of the fifteen siblings of two positive parents when only a minority would be expected. This is further corroborated by the frequent absence of rheumatic fever in both parents in many families. The line-by-line agreement in the distribution of families with one, two, three, etc. affected siblings in the Appendix, Table IX, does not support the assumption of the mechanism of irregular dominance. The agreement with the theoretic binomial distribution in the Appendix, Table IX, is also not consistent with this assumption.

Sex Linkage.—Among the progeny of positive mothers by negative fathers, 60.0 per cent of seventy daughters were positive and 50.7 per cent of seventy-five sons were positive (Appendix, Table XII). If sex linkage were involved, no positive daughters should occur in this series, although 50 per cent of the sons should be positive. In the appendix, Table XII, the analysis of the data for matings of two negative parents is shown. The occurrence of approximately 50 per cent of positive siblings of both sexes is consistent with the hypothesis of autosomal recessive inheritance and inconsistent with an hypothesis of sex linkage. Dominant sex linkage need not be considered for reasons analogous to those for which autosomal dominance was excluded.

Furthermore, in thirty-five matings of rheumatic mothers with normal fathers, somewhat more than one-half of the daughters as well as the sons were found to be rheumatic, although all the sons and none of the daughters would be expected to be rheumatic if sex linkage were involved. In the four matings where both parents were rheumatic, as well as the fifty-nine matings where neither parent was rheumatic, the ratios are equally inconsistent with the hypothesis of sex linkage. The phenomenon of partial sex linkage recently discovered by Haldane[33] could not be tested in our data.

Draper has suggested that a sex-linked factor may be responsible for the susceptibility to rheumatic fever. Examination of his data, however, reveals that the reported excess of affected females is eliminated if the index case is disregarded, since a disproportionate number of index cases were females.[34]

Complementary Recessive Genes.—The analysis for two complementary recessive genes gave results which were inconsistent with this hypothesis. Similar results were obtained using the Lenz equation ($p = 1/16$). An alternative test for a complementary recessive gene mechanism was made. The occurrence of multiple case families was compared to the expectation for various values of p.

As the family size increases, the number of multiple case families increases. On a multiple gene hypothesis, it would be expected that the rate of increase of multiple

case families would be lower than the rate of increase for a single gene hypothesis. Table XIII of the Appendix shows that the rate of increase is consistent with the trend expected for a single recessive gene and inconsistent with the expectation on the hypothesis of two recessive genes. Similar estimates were made for three and four recessive factors, and disparity was even greater between prediction and observation than for the two-factor hypothesis.

Complementary Dominance.—Hogben[32] has called attention to the necessity for differentiation between a single recessive gene and two or more complementary genes for data which show good agreement when analyzed by the Lenz formula. Analysis for multiple complementary dominant genes may be made for relatively rare traits by substituting appropriate values for p in the Lenz formula ($p = 0.125$ and 0.0625 for two and three genes, respectively) in matings of two negative parents. In our data this analysis was inconsistent with the interpretation of a complementary dominant gene mechanism. However, this method is not sufficiently critical to exclude a complementary dominant gene mechanism in a disease as prevalent as rheumatic fever.

Another test of complementary dominant gene interaction may be made when the prevalence of the disease in the population is known. The relative frequency of two genes may then be computed. In rheumatic fever, however, since the prevalence in the general population is not known, the test is made indirectly. The frequencies of the two genes have been assumed to be equal and by trial the most probable values for the gene frequencies found, based on agreement between observed and computed values. Table XIV of the Appendix shows that agreement is best when each gene has a value of 0.1576. There is no direct test for the accuracy of this value, although it may be used to compute the theoretic prevalence in the general population. The theoretic prevalence of rheumatic fever was found to be 8.4 per cent by this method. This exceeds any estimates so far recorded for clinically recognizable rheumatic fever in the general population. On this basis the hypothesis of complementary dominant genes is not sustained. However, with additional assumptions regarding the relative frequency of the two genes it may be possible to arrive at better agreement. This was not attempted because the achievement of a satisfactory agreement would not warrant any definite conclusions in view of the number of additional assumptions involved.

A direct test of the two gene hypotheses could be made with matings of two rheumatic individuals or of a rheumatic with a nonrheumatic parent in which selection on the basis of one rheumatic sibling was not practiced. Families of this type are now being studied.

Extensions of Genetic Techniques for Simultaneous Genetic and Epidemiologic Analysis

It has been shown that when the majority of the siblings in this series of rheumatic families have reached or passed the age-susceptible period, genetic predictions of *final case incidence* are satisfactorily realized. If the estimates of case incidence are made in earlier calendar years, it is apparent that all of the cases predicted will not have had an opportunity to be realized. Such a prediction therefore represents an estimate of *the number of genetically susceptible* individuals present in the families at the time of analysis. For purposes of epidemiologic analysis it was necessary to estimate the number of susceptible individuals present in the families at various times during their life experience.

Predictions in Arbitrary Calendar Years.—Analyses were made in six arbitrary calendar years: 1924, 1927, 1930, 1933, 1936, and 1939.

Estimates were made using the genetic factors (Table IV). The families were arranged according to family size in each calendar year, and all individuals present at the given date were included. (A few families were excluded in the early calendar years since they did not meet the requirements of the Lenz-Hogben formula, that is, among their numbered offspring at the time of analysis no cases were ever realized.) The estimates were compared to the actual number of cases finally observed (1939) among the *same* individuals.

In Table VI it may be noted that in the early calendar years expectation was not fully realized, although final case incidence was adequately estimated in every calendar year. This finding indicates that there were other factors responsible for the onset of rheumatic fever among susceptible individuals.

TABLE VI

CASES EXPECTED IN SIX CALENDAR YEARS AND FINALLY OBSERVED AMONG THE SAME SIBLINGS

YEAR	BOTH PARENTS NEGATIVE				ONE PARENT POSITIVE			
	NUMBER OF FAMILIES	CASES EXPECTED	CASES FINALLY OBSERVED	OBSERVED IN CALENDAR YEAR	NUMBER OF FAMILIES	CASES EXPECTED	CASES FINALLY OBSERVED	OBSERVED IN CALENDAR YEAR
1924	48	63.79	64	16	39	67.07	70	24
1927	57	78.65	86	37	48	87.57	94	43
1930	59	84.96	97	64	50	99.16	113	68
1933	59	88.28	101	85	50	106.52	117	92
1936	59	89.90	101	97	50	111.07	117	114
1939	59	89.90	101	101	50	112.04	117	117

Predictions at the Time of Onset of the Primary Case.—Prediction of the number of susceptible individuals present at the time of onset of the primary case were also made, using a similar method of analysis. The families were grouped according to family size in the year of onset of the first case. In Table VII it will be observed that the prediction made at the time of onset of the first case was fully realized at the time of final observation (1939). It may therefore be stated that all susceptible children who were present in the families at this time were adequately estimated by this method. It is also apparent that at the time of onset of the primary case, the entire expectation was not realized, indicating the operation of other factors.

Predictions Following the Onset of the First Case.—The techniques used in the preceding analyses cannot be used for estimating the number of susceptible individuals present in the sample after one case has been excluded, since the sample then does not conform to the criteria of the Lenz-Hogben equation; that is, the residual sample does not include at least one case per family. However, it is suitable for the use of simple genetic ratios.

TABLE VII

CASES PREDICTED AT THE TIME OF ONSET OF THE PRIMARY CASE AND FINALLY OBSERVED

	SIBLINGS AVAILABLE	CASES EXPECTED	OBSERVED	
			AT THE TIME OF ONSET OF THE PRIMARY CASE	TOTAL
Both parents negative	190	78.5	60	83
One parent positive	152	88.7	50	92

TABLE VIII

EXPECTATIONS IN THE PERIOD FOLLOWING THE ONSET OF THE PRIMARY CASE ACCORDING TO FAMILY SIZE IN NEGATIVE PARENT FAMILIES (1939)

ORIGINAL FAMILY SIZE	SIBLINGS AVAILABLE	EXPECTED	OBSERVED
1	--	--	-
2	10	2.50	3
3	28	7.00	6
4	35	8.75	6
5	38	9.50	8
6	25	6.25	7
7	19	4.75	7
8	28	7.00	4
9	8	2.00	-
Total	191	47.75	41

TABLE IX

CASES PREDICTED AND OBSERVED IN SIX CALENDAR YEARS FOLLOWING THE ONSET OF THE PRIMARY CASE

	SIBLINGS AVAILABLE*	TOTAL CASES EXPECTED	CASES OBSERVED IN CALENDAR YEAR	CASES OBSERVED LATER	OBSERVED TOTAL
Both Parents Negative					
1924	44	11.0	2	7	9
1927	86	21.5	6	19	25
1930	138	34.5	19	15	34
1933	181	45.5	26	15	41
1936	191	47.8	37	4	41
1939	191	47.8	41	--	41
One Parent Positive					
1924	38	19.0	12	12	24
1927	67	33.5	19	18	37
1930	115	57.5	28	27	55
1933	136	68.0	45	20	65
1936	153	76.5	64	3	67
1939	155	77.5	67	--	67

*Includes only siblings in families in which one case has already occurred.

It has been shown that the original sample follows the binomial distribution with the exception that there are no families present without any cases (Appendix, Table IX). When the first case is excluded, this bias is eliminated and the distribution of families is in accordance with the complete binomial distribution (Appendix,

Table VIII, A), provided that the families are tabulated according to their *original* family size, regardless of the exclusion of the first case.

When the distribution of cases in each family size is described by the *complete* binomial distribution (Appendix, Table VIII, A), the simple ratios of 0.25 and 0.50 for negative-by-negative and positive-by-negative families, respectively, may be used for obtaining a prediction of secondary cases in the sample.

The use of the simple ratio is not always possible in clinical material, since there is often an excess of multiple case families in the usual clinic sample. The preliminary testing with the binomial distribution will permit a decision as to whether this technique may be used.

Simple genetic ratios were applied to the data after the primary case was excluded. In Table VIII is presented a comparison, according to original family size, of cases predicted and observed in 1939. The satisfactory agreement obtained demonstrates the validity of this method for predicting secondary cases.

Using the simple ratios described, it is possible to predict the number of susceptible siblings present who should subsequently be realized as secondary cases. This was done in six arbitrary calendar years. In Table IX it may be seen that there is good agreement between expectation for secondary cases and final observation. In other words, the genetic susceptible individuals present in the sample at any time following the onset of the primary case may be predicted by this method. It will again be noted that particularly in the earlier calendar years expectation was not realized.

Individual Expectancies.—In the preceding genetic analyses, predictions were made using the *family* as the unit for study. For purposes of epidemiologic analysis, it would be desirable to use the *sibling* as the unit for study so that each child could be followed individually throughout his life experience.

One method which is suitable for some purposes is to divide the appropriate Lenz-Hogben factor equally among all the siblings in the family. (Similar computations may be made with the Bernstein equation or with other formulas for appropriate genetic situations.) This method gives each sibling an equal genetic chance to be susceptible, although it is obvious that all siblings are not equally susceptible.

In order to test the validity of this procedure a comparison was made of predicted and observed cases in thirty families with ninety-nine siblings (of both mating types). Predictions were made using the family as a unit, and by the method of assigning individual expectancies to every member of the sibship. The computation was restricted to siblings over 14 years of age because they had passed the maximum age of onset in our sample. The agreement which was obtained between prediction and observation by both methods indicates that equal individual expectancies may be assigned to each sibship (Appendix, Table XV).

It was also found that under certain circumstances the Lenz-Hogben factor may be divided unequally among all siblings in the family. The

usefulness of this procedure for integrating genetic and epidemiologic analyses is illustrated in the subsequent presentation.

Comment

There are a number of possible sources of error in the genetic analysis of human family data which must be evaluated. The incomplete ascertainment of cases is probably the most important factor, for diagnostic errors are apt to occur in rheumatic fever. The disease is often insidious and subclinical and is frequently overlooked. Since its usual onset and characteristic manifestations occur during childhood, adults frequently do not recall their earlier experiences. Unless demonstrable rheumatic heart disease is present, the diagnosis frequently cannot be made. For this reason it is not advisable to study families in which some members, the children, are seen during the critical years of the disease, while others have entered into the study in adult life through history of aunts, uncles, and grandparents. Fortunately, in our data the prolonged observation of the families under the same medical supervision and restriction of the analysis to the immediate household has made the sample more homogeneous and has minimized errors in diagnosis.

The limitations of historic data in rheumatic fever have been fully discussed. It must be emphasized that if the diagnostic errors in historic records are numerous, conclusions from such data cannot be made. Of particular significance is the importance of an accurate parental diagnosis in hereditary diseases. An error in a parent is disproportionately greater than a similar error in a sibling. When a genetic factor is involved, an error in parental diagnosis would lead to an excess of cases in those families where both parents were defined as negative. The small excess of cases noted in our negative parent families has been attributed to a few errors in parental diagnosis. If a consistent excess or deficiency of cases occurs in each family size, it may be attributed to diagnostic criteria utilized, provided that the operation of nongenetic factors is excluded.

Tabulations of sibships in genetic studies often reveal large fluctuations within the data. In our data the consistently small deviation for fraternities of different sizes in all of the analyses indicates that the sample is uniform.

There are a number of other factors which may cause the observed familial incidence of hereditary disease to fall short of the frequency postulated on genetic grounds. Among these are lesser viability of the disease type, wide variations in the age of onset as in diabetes, and variation in the expresssion of the condition which leads to overlapping of the normal condition. Of particular importance are possible nongenetic effects which must be evaluated explicitly.

In our studies nearly all of the families have been followed from an early part of the reproductive history of the mother, and the majority of the siblings were past puberty when the final analyses were made.

Much of the data in this study is not fully comparable to other published family studies because of differences in the criteria of selection of families. In some studies cases among parents and children are not differentiated. In others the requirement of at least two cases in every household insured the inclusion of two siblings when both parents were normal and one when one parent was rheumatic and none when both parents were rheumatic.

The process of fitting a genetic hypothesis to a set of data is not in itself adequate proof of the validity of that hypothesis but only states that the hypothesis is not inconsistent with the data.

It might be argued that nongenetic factors may be exclusively responsible for the familial concentration of cases, agreement with a specific gene mechanism occurring by chance. This possibility has been evaluated in many diseases in which heredity is accepted. Fortuitous agreement may be excluded by testing the genetic hypothesis on various subdivisions of the data. It has been demonstrated that good agreement between observed and predicted values was obtained in numerous subdivisions of our data. It would seem reasonable to conclude that the agreement with the genetic postulate which was obtained in our data is not due to chance.

The hypothesis of heredity is enormously strengthened for a given condition if it can be shown to fulfill the detailed requirements of any known mechanism of inheritance. The statistical distribution of cases may be explicitly stated for various degrees of kinship of a family with respect to dominant and recessive genes, sex-linkage, and other more recondite genetic relations. We have analyzed our data with respect to each of the more important gene mechanisms and have found that the theory of a single recessive gene is adequate to describe the familial distribution of cases as it finally occurs, while the other mechanisms fail in various respects.

There was no agreement when mechanisms such as a single dominant gene, sex-linkage, two or more recessive factors, or more than two dominant factors were postulated. Although the operation of a double dominant gene was not entirely excluded, application of this hypothesis to the data gave an estimated frequency of 8 per cent for the disease in the general population, a value far higher than is estimated by current observers.

The assumption that the mechanism is a single autosomal recessive gene is consistent with the data and with the general observed incidence of the disease. It is also the simplest concept which may be postulated. It was therefore felt that for purposes of further analysis, this genetic mechanism should be assumed, and, if correct, the results would substantiate it.

It is recognized that the interpretation of a single recessive gene may prove inadequate as an explanation of the data and that a more complex hypothesis of genetic transmission may be required. In that event, ap-

propriate expressions may readily be derived from whatever hypothesis proves to be more suitable. The analyses which have been made would remain useful as approximations, failing only in those critical matings where there were divergent predictions on the two hypotheses.

For the present time, the argument for heredity rests on the formulas which have been used and the adequacy with which they describe the data.

In this presentation many statistical procedures were necessary in order to establish the probable mechanism of inheritance in rheumatic fever. Genetic techniques were extended to permit integration of genetic and epidemiologic analyses. The actual methods for the genetic analysis of data are relatively simple and have been presented in a form which should facilitate comparable studies.

(References will appear at the end of Part II—conclusion of this study.)

Erratum

On page 235 of the February issue of the JOURNAL in the article by H. A. Agerty, M.D., A. J. Ziserman, M.D., and C. L. Shollenberger, M.D., entitled "A Case of Perforation of the Ileum in a Newborn Infant With Operation and Recovery," the first line of the case report should read as follows: M. R. was a four-week premature white male infant weighing 5 pounds, 12 ounces at birth.

The Pediatrician and the War

On March 8 the following information was released from the Office of Price Administration which is of interest to pediatricians and answers a question that has been frequently asked.

Additional allowances of rationed baby foods may be obtained for a child by the parent presenting a statement signed by a licensed physician, stating why the present allowances are insufficient for the child's diet, how much more processed foods he needs, and why fresh and unrationed foods cannot be used to supplement the rationed.

For example, it would require 60 points for a can of vegetables and a can of fruit each day, while the child's allowance is only 48 points.

Evaporated Milk

So many questions have been received in regard to the supply of evaporated milk for infant feeding purposes that the JOURNAL has obtained the following information which it believes can be considered authoritative.

In the past five years the average civilian consumption of evaporated milk was about 50 million cases per year, each case containing 100 pounds, or 46 quarts, of fluid milk. The War Production Board has allocated sufficient tinplate for an anticipated production in 1943 for the evaporated milk industry of about 75 million cases. This amount can be achieved if the raw milk supply holds out.

The estimated needs for evaporated milk for war purposes, including the requirements of the armed forces, Lend Lease, Red Cross, etc., is about 40 to 45 million cases for 1943. About 18 million cases were in the hands of the government on January 1, and so an additional 25 million cases would be required from the 1943 production. There will be left available, therefore, for 1943 approximately the same amount of evaporated milk for civilian consumption as has been consumed on an average during the past five years.

One of the important causes for the recent shortage of evaporated milk has been due to excess buying over and above the actual needs of housewives. This has recently tapered off, and grocers are accumulating better stocks. Should either another wave of hoarding take place or the production for any reason fall below the estimated goals, rationing of evaporated milk will probably result in order to provide for an equitable distribution among the people. Pediatricians can be assured, therefore, that in all probability normal supplies of evaporated milk will be available during 1943. Although rationing would not seem to be necessary, if it should come, adequate provision would be made for the needs of infants and children.

American Academy of Pediatrics

Proceedings

ELEVENTH ANNUAL MEETING OF THE AMERICAN ACADEMY OF PEDIATRICS

BOSTON, MASS.—OCT. 9-11, 1941

Panel Discussion on the Vitamin B Complex

Chairman: Frederick T. Tisdall, M.D., F.R.C.P.(C), Toronto, Can.

Associates: Horton Casparis,† M.D., Nashville, Tenn.
Theodore G. H. Drake, M.B., F.R.C.P.(C), Toronto, Can.
Norman Jolliffe. M.D., Associate Professor, New York University College of Medicine, New York, N. Y.
W. H. Sebrell, Jr., M.D., Chief, Division of Chemotherapy, National Institute of Health, U. S. Public Health Service, Bethesda, Md.

CHAIRMAN TISDALL.—In 1912 vitamin B_1 was found, or a concentrate was found which we now know contained thiamine or vitamin B_1. In 1926 it was found there were two fractions, one that was destroyed by heat and the other that was not. The one that was destroyed by heat is now known to be thiamine. The other fraction has been divided into a number of different members.

In 1933 riboflavin was discovered. In 1938 Henry Sebrell was the first to demonstrate that it was necessary for human nutrition.

In 1940 Sydenstricker, Kruse, and Sebrell showed that certain vascular changes in the eye occurred when this vitamin was lacking in the human diet, and a simple technique was worked out to demonstrate these changes.

The action of nicotinic acid was discovered in 1937. Elvehjem showed that nicotinic acid would cure black tongue in dogs, and within two or three months three or four workers showed that it would cure pellagra in human beings.

Then there is pyridoxine, which was discovered two or three years ago, and in 1940 Spies showed that fatigue in human beings was cured by the administration of pyridoxine. Jolliffe showed its effect in the treatment of paralysis agitans.

The fifth one is pantothenic acid, and before long it will probably be shown to be necessary for human nutrition. In the past year other members of the vitamin B complex have been discovered.

We are going to discuss these substances from the standpoint of the practicing pediatrician.

First, Dr. Sebrell will tell of our approach to all these members of vitamin B complex from the national standpoint, not from the standpoint of our individual patients, and take a minute or two to bring home to us the fact that we are dealing with a number of different vitamins and the fact that if we see evidences of a lack of one there will probably be a lack of some of the others also.

†Deceased.

DR. SEBRELL.—Our national nutritional program is developing as an unexpected result of the National Nutritional Conference which was held in Washington in May. Originally we expected an attendance at that conference of somewhere around 400 or 500. We wound up with more than 900 people there, who paid their own expenses to come to Washington for that conference. There was a great deal of interest displayed, and a number of recommendations were made to the Government as a result of that conference.

We are now trying to follow up these recommendations, and the office of the Director of Health, Welfare, and Related Defense Activities, which Mr. McNutt heads, has been divided into two principal divisions: (1) recreation, headed by Mr. Taft, and (2) nutrition, headed by Director Wilson and myself.

We are attempting to do something in the line of coordination of all the various agencies that are interested in nutrition in this country. State nutrition committees have been set up in each state, and under those state nutrition commitees there are hundreds of county nutrition committees and a few local nutrition committees.

It is really essential that the pediatricians and the medical societies take an active part in the activities of these nutrition committees if we are to get anywhere. In the past most of the nutrition work in this country has been done by home demonstration workers, extension department workers, and people interested in agriculture, and there has been little done on the medical side. Some state health departments and some of the city health departments have been interested.

We are not attempting to put into effect a new program. What we want to do is to coordinate the interested agencies all the way from food production to its utilization and its effect on health and well-being. We are trying to fit these state and local committees into state and local defense councils. We feel they should be subcommittees of the defense councils and as such they will utilize the various workers in the different communities to carry out the details of this program. I want to urge all of you in your communities to take an active part and see that the medical profession, the pediatricians, and the health departments are properly represented on these committees, so that the health and medical side of these committee activities may go in the right direction.

There is a big job for you to do, and whether it is done right or not depends to a large extent upon the medical cooperation in the program.

I want to conclude by urging all of you to take an interest in these activities.

The Federal agency is not going to set up any organization below a regional level. That is certainly as far as we will go in order to service these state and local activities. It is up to the states and the communities themselves to solve their problems. And it must be more than just an educational approach to the problem. Education is important and it plays an important part, but it does not get at the part of the problem which is economic, the problems of production and distribution. We need to devise means of getting proper foods to the people who need them. Much can be done in that field. With your help it can be done properly and efficiently.

QUESTION.—Do you ever get a pure nicotinic acid or thiamine deficiency?

DR. SEBRELL.—That is a large order to cover in a few minutes.

Let us turn from the national program to the clinical problems. In the clinic it is of course exceptional to see a single pure deficiency disease. In the B complex deficiency, and with the other vitamin deficiencies, for that matter, what you usually see is a multiple deficiency. Assuming that the cause is inadequate intake, the diet on which these people develop these diseases are not selected diets, as we would select an experimental diet. If it is deficient in one thing, it is likely to be deficient in another. Usually the symptoms of one deficiency tend to predominate, although the symptoms of two or three deficiencies are apparent at the same time and are ex-

tensively present. However, you may have a condition in which you see symptoms of thiamine deficiency and you see no evidence of nicotinic acid deficiency or riboflavin deficiency. If you make a diagnosis of thiamine deficiency and treat that patient with thiamine, the other deficiencies become apparent. You may see an individual who appears to have a pure thiamine deficiency. If he is given nothing but thiamine and his diet is restricted in the other members of the complex, such an individual will develop riboflavin deficiency, nicotinic acid deficiency, or both, while in the hospital.

A patient will come in with the typical skin lesions of pellagra, and if he is treated with nothing but nicotinic acid and his appetite is poor and he does not eat the hospital diet, not infrequently in a few days or a week or two he turns up with symptoms of riboflavin deficiency.

The multiple thing is what is usually seen in the clinic, and it is just a matter of separating out these lesions. Being able to distinguish which deficiency exists enables you to make the multiple diagnosis. You can separate them out therapeutically if you are careful to restrict the diet and give just the pure substances.

If you have a patient with the skin lesions of pellagra, the eye lesions of riboflavin deficiency, and the peripheral neuritis of thiamine deficiency, you can hold his diet down and give thiamine and his peripheral neuritis will clear up, but his skin and eye lesions will continue and you can clear them up separately.

Therefore, in treatment it is important to give large amounts of the material which is needed to cure the symptoms which are the most prominent. At the same time do not forget it is essential that the individual either have a very good diet at the same time or that the other members of the B complex should also be given as a supplementary treatment to the specific symptoms which seem to dominate the picture at that particular time.

CHAIRMAN TISDALL.—How frequently are these deficiencies due to poor absorption?

DR. SEBRELL.—I cannot say how frequently they are due to poor absorption. You must recognize that a number of cases occur in individuals who are receiving what appear to be adequate diets.

You can get deficiency diseases from causes other than deficient intake. There may be faulty absorption, especially if there is some disease of the gastrointestinal tract which speeds up the time of the passage of food through the tract or interferes with the absorption or digestion of the food which is taken in.

We see a number of cases of that kind which occur secondarily along with such things as liver disease, carcinoma of the gastrointestinal tract, colitis, and various gastrointestinal diseases which interfere with absorption. They may occur where there is metabolic disease or interference with utilization. We see them complicating diabetes.

I saw a patient recently who came for a severe diabetes with no obvious deficiency disease. That patient was given insulin and glucose and overnight developed the typical skin symptoms of nicotinic acid deficiency. From these other causes you may have a deficiency disease in spite of adequate food going into the mouth.

I saw another patient who developed a severe vitamin A deficiency in spite of a daily intake of 50,000 units. Putting the stuff in the patient's mouth is not enough. That patient had an intestinal disease which caused a failure to absorb this large dose of vitamin A.

These patients that I speak of form a large group.

I should mention here the increasing attention which is being given to the use of these vitamins in surgical cases, where glucose and saline solutions are given parenterally. If you think about what the surgeon is doing there, you can see why

such treatment without adequate vitamin administration occasionally produces deficiency disease in that patient. By giving large doses of saline they are washing out the vitamins that remain in the tissues. At the same time the patient is not given anything to eat. By giving glucose you push up the metabolism and the utilization of those vitamins which are necessary, without replacing them. As a result the suspicion is growing that much of the disability and possibily part of the mortality following surgical operations is due to this effect on a patient with a low vitamin reserve at the time of the operation. In giving glucose and saline parenterally it is quite important that the patient receive thiamine at the same time parenterally, and you can inject the thiamine into the rubber tube through which the glucose is being administered. It is quite important that that be done. It will make a difference in the postoperative course of your surgical operations.

CHAIRMAN TISDALL.—I gather that you believe we are apt to get a deficiency of two or three of these B complex elements?

DR. SEBRELL.—That is right.

CHAIRMAN TISDALL.—These patients are a very scientifically treated group of people, and many of us give thiamine to our patients. The only reason a pediatrician would give thiamine would be after he had made a very definite examination of that patient and had come to the conclusion, as the result of his scientific examination, that the patient needed thiamine.

That being so, should he not hunt around to make sure there were not signs of riboflavin deficiency and nicotinic acid deficiency?

DR. SEBRELL.—The pediatrician should not be disappointed if thiamine alone does not clear up that patient's condition right away.

DR. DRAKE.—Why not administer all the vitamins? Why stop at giving thiamine only?

DR. SEBRELL.—You should know what is the matter with your patient before you begin giving him things. I personally like to make a definite diagnosis and have the satisfaction of knowing where I am going with my therapeusis when treating these specific conditions. However, there is more than that involved.

If the predominating symptoms are of one condition, as is frequently the case, that condition needs intensive treatment. If you have a case of thiamine deficiency, unless you give a pretty good dose of thiamine the recovery is going to be quite slow, and when you give the vitamin shotgun, if you follow the course of most physicians, you will grab some proprietary preparation that a persuasive drug man has indicated is pretty good, and most of those which are made up of a mixture of vitamins do not contain those things in therapeutic doses. There are a few that contain an adequate daily supply of each of the vitamins. Most of them are definitely inadequate from the point of view of normal daily supply in some of the vitamins and contain too much of others. For therapeutic purposes they do not contain enough of any one of them. I refer to the multiple preparations, a few of which I am familiar with. You need to give enough of the material for therapeutic purposes and more than you can find in the usual mixture of vitamins that are being sold today. That is one reason for giving large doses of these specific ones.

DR. DRAKE.—You believe that to treat these deficiencies the physician should build up a prescription containing the required amount of the different vitamins, just as carefully as he builds up a prescription to treat anything else, and not simply order four ounces of so-and-so's elixir of multiple vitamins?

DR. SEBRELL.—That is the way I look at it.

CHAIRMAN TISDALL.—We will now discuss the individual deficiencies, starting with thiamine, then riboflavin and nicotinic acid.

I will ask Dr. Jolliffe to discuss this on the basis of some of the symptoms of thiamine deficiency, some of the requirements, and the treatment. We are discussing all this now from the therapeutic aspect. Later we will discuss the preventive aspect.

DR. JOLLIFFE.—I like to think of the symptoms of thiamine deficiency as they have been isolated from the group of symptoms resulting from multiple deficiency in somewhat the following terms: Nutritional failure begins the instant the internal environment fails to receive an adequate amount of an essential nutritional element. This failure may be due to failure to ingest, to absorb, to utilize, or to retain these elements. This results in tissue depletion, and that is followed in succession by biochemical lesions, functional changes, and finally by anatomic changes. The recognition of the symptoms produced by these changes follow, as a rule, the reverse order.

Let us take the example of thiamine deficiency. For decades the clinical picture of beriberi has been recognized. Beriberi was at first thought to be rare in this country, in spite of the fact that we had identical clinical pictures in a great number of conditions. These clinical pictures consisted of polyneuritis or polyneuropathy, associated with various diseases that increased the requirement, prevented the absorption, or impaired the utilization of the vitamins. For example, we had patients with polyneuritis occurring in gastrointestinal diseases, in alcoholism, in pregnancy, in infectious diseases, and in cachexia.

These conditions usually were designated as alcoholic, gastrogenous, infectious, or cachectic polyneuritis. A great number of these are now recognized as actual beriberi, or thiamine deficiency.

If we are to look for a complete picture of polyneuritis, it is, of course, a rare disease; that is, a clinical picture with bilateral foot and wrist drop and paralysis of the extremities. Like heart failure, there are all degrees of polyneuritis.

The earliest objective signs of polyneuritis are plantar dysesthesia and calf muscle tenderness. I do not believe it is possible to make a diagnosis of thiamine deficiency on these two signs. But when these two signs are present plus loss of ankle jerks, plus impaired vibratory sensation in the toes, it is then possible to make the diagnosis of polyneuritis.

With the average internist and surgeon, I will not say the pediatrician, the neurologic examination consists in doing the pupillary responses and knee jerks and possibly a Romberg test. These tests will show most subjects having thiamine deficiency.

We recently recognized that the bilateral ophthalmoplegia of Wernicke's syndrome is a thiamine deficiency. These patients usually have severe diarrhea, or alcoholism, or tuberculosis of the intestinal tract. They develop a polyneuritis, and superimposed on that they develop a clouding of consciousness, ataxia, and bilateral ophthalmoplegia. This ophthalmoplegia does not respond to any of the crystalline B vitamins other than thiamine. Give these patients adequate doses of thiamine and they will respond very dramatically within forty-eight to seventy-two hours. So much for the anatomic lesions.

We noticed that many of these patients with objective signs of polyneuropathy had various symptoms that are usually called neurasthenic. They had gastric disturbances, fatigue or fatigability, and disturbance of sleep plus a lot of other queer bodily sensations. We noted in treating these patients that a great number of these symptoms cleared along with the objective signs of polyneuropathy. We attributed it to better nutrition plus psychotherapy. We then induced an isolated thiamine deficiency in normal subjects and found that they developed these same neurasthenic symptoms. These symptoms, too, cleared up on supplying thiamine to the diet. The Mayo group have reproduced by isolated thiamine deficiency similar symptoms that they were able to relieve by thiamine.

We now come to the chemical lesions. Following the development of a method for the accurate determination of pyruvic acid in the blood by using iodoacetic acid as a stabilizer, we found in the normal subjects, under basal conditions, that the fasting level was about 1 mg., with an upper limit of normal of not over 1.3. In our thiamine-deficient subjects they often had an elevated fasting level, but not always. We then put normal subjects on metabolic strain by giving them 1.75 Gm. of glucose per kilogram and studied the pyruvic acid for the next five hours. In the normal subjects the maximum rise occurs at the end of an hour but returns to the fasting level at the end of three hours.

In our thiamine-deficient subjects, patients having acute polyneuropathy or Wernicke's syndrome, the pyruvic acid continues to rise after the first hour and does not return to normal within three hours. The pyruvic acid curve would return to normal when these subjects were treated with thiamine.

QUESTION.—Are the members of the B complex water soluble or fat soluble?

DR. CASPARIS.—Water soluble.

QUESTION.—Tell us about the therapeutic dosage of thiamine. We have made the examination including the ankle and the knee jerks. We have diagnosed that this child is suffering from lack of thiamine. What are we to do?

DR. JOLLIFFE.—In adults the first thing to do is to give as good a diet as it is humanly possible to provide. That does not mean placing the diet by the side of the bed and trusting that the patient will eat it. We emphasize diet for the reason that it is impossible at the present time to "ampoulate," encapsulate, or tabulate all the vitamins.

We then give the patient vitamin B complex from a natural product such as yeast or liver or their concentrates. We usually give these in fairly large doses. For example, of brewer's yeast we usually give three ounces daily. Of crude liver water extract we give about one-half that amount, and of yeast extracts, about one-third of that amount. These supply, in addition to the diet, many of the unknown factors as well as many of the known factors of the B complex. By this method we feel reasonably certain that our patients are ingesting all the B vitamin factors.

Then, if the patient has clinical evidence of thiamine deficiency, we give thiamine. If there is clinical evidence of riboflavin deficiency, we give riboflavin, and so on down the line. For thiamine deficiency we usually give between 10 and 50 or on occasion 100 mg. of thiamine a day.

If we think the deficiency happens to be in absorption, we give thiamine parenterally. Even in utilization or in liver diseases it may be given parenterally. It requires about five times the prescription or recommended adult allowance of thiamine.

CHAIRMAN TISDALL.—If a baby was on a 1,000-calory diet, you would put it on one-third the adult dose?

DR. JOLLIFFE.—Yes, approximately one-third.

On the basis of our experimental study of actual clinical but mild deficiency, good results are obtained on about 10 mg. of thiamine; with riboflavin about the same amount. These are about five times the recommended daily allowances.

I am not able to say whether 20 mg. of riboflavin would hasten the improvement. I know that ordinarily they will improve dramatically and quickly on 10 mg. daily by mouth, in addition to what you include in the diet.

For nicotinic acid the satisfactory daily allowance is somewhere in the neighborhood of 20 mg. We ordinarily give 500 to 1000 mg. of nicotinic acid in divided doses for curative results.

CHAIRMAN TISDALL.—This particular part of the information you have given is so important, that I am going to ask Dr. Sebrell to give his views on exactly the same thing.

DR. SEBRELL.—We do not have any serious disagreement in our therapeutics. Dr. Jolliffe's dosage of nicotinic acid I would regard as being a little bit high. With that quantity of nicotinic acid you are certain to get reactions. Those reactions are harmless but they frighten the patient unless you have warned that they are going to occur.

With 200 or 300 mg. of nicotinic acid to a dose, you get shortly following the administration, within five or ten minutes, a burning sensation of the skin; the patient looks as though he is blushing all over. Sometimes it affects different parts of the body. Sometimes the entire body is involved and sometimes not. There is this feeling of heat in the skin, which is rather uncomfortable. You never get those reactions with nicotinic acid amide, which has the same therapeutic effect as nicotinic acid.

We have not talked about the clinical picture of nicotinic acid deficiencies. Dr. Jolliffe did one of the finest pieces of work that has been done in restoring nicotinic acid deficiency in encephalopathy which occurs without the skin lesions. That condition, if I interpret his writing correctly, does require larger doses than the usual skin lesion type.

In that type of deficiency I believe you need the very large doses in contrast with the dose required in the usual skin lesion type.

DR. JOLLIFFE.—The symptoms we have described as nicotinic acid deficiency encephalopathy occurs in several groups of patients. The principal one is the alcoholic, particularly those who have delirium tremens. But it also occurs in senility and cirrhosis of the liver, and postoperatively.

The symptoms are stupor associated with changing cogwheel rigidities of the extremities, grasping reflexes, and sucking reflexes. I like to compare the sucking reflex to little birds in a nest when the mother approaches with a worm, and as the mother moves from one side to the other the little birds will follow the mother with mouth wide open. In the patients, when you start to place a tongue depressor near the mouth they will purse the lips, and if you move the depressor from side to side they will follow it with pursed mouth.

We thought at first that this syndrome was a dehydration phenomenon and we treated them by rehydration. I think in about sixty patients we had fifty-eight deaths.

Along about that time thiamine became available and, as a number of these patients had thiamine deficiency polyneuropathy, they were given large doses of thiamine, about 100 mg. daily. In fifteen patients we had fifteen consecutive deaths. We then thought, since many of the patients had signs of pellagra, that we would give them the entire B complex. By this method we were able to reduce the mortality from 95 per cent to around 60 per cent. It was not enough to be particularly significant.

When nicotinic acid became available, we tried it in fairly large doses, 100 mg. doses about ten times a day, and our mortality was reduced to about 30 per cent in our first group of patients. Since that time we have been able to reduce the mortality in a group of over a hundred patients with nicotinic acid plus the whole wheat complex to about 15 per cent.

CHAIRMAN TISDALL.—We have not discussed the symptoms that occur as the result of riboflavin deficiency. Dr. Sebrell was the first to show the need of riboflavin for human metabolism.

DR. SEBRELL.—The symptoms of riboflavin deficiency syndrome may be conveniently divided into three groups: (1) the ocular symptoms; (2) the oral symptoms; and (3) the skin lesions.

In regard to the ocular symptoms, the earliest change that we see in the eye in riboflavin deficiency is a growth of capillaries into the cornea. These fine capillaries cross the sclerotic and go into the cervix of the cornea. They can be seen only by the slit lamp microscope. It requires a corneal microscope in order to see those vessels steadily enlarge and proliferate into the cornea until you get a lesion which you can see grossly and which on superficial examination appears to be a conjunctivitis. There is photophobia and there is interference of vision. The photophobia might be quite intense so that the individual cannot bear light at all. In some of them we cannot get the eyes open to examine them because of the pain. The vessels of the conjunctiva are intensely engorged. As the lesion progresses you get cloudiness of the cornea and then you can see very obvious opaque spots in several of them, and in some instances corneal ulcers may occur.

We have seen several patients with a complete ground-glass cornea with a cataract of blood vessels across the cornea, where the vision was reduced to practically nil. Following the use of riboflavin these lesions in most instances have completely disappeared. The corneal opacity goes away and the individual returns to nearly normal vision.

If the lesion is of several years' standing and the vessels have clouded and the corneal opacity is dense, you cannot expect that sort of thing to disappear by supplying riboflavin deficiency. In these acute lesions remarkable improvement has taken place in a few days or a week or two. Overnight you get a marked relief of the subjective symptoms about the eye and you find the photophobia is lessened, although you cannot see any obvious change on gross examination. With the slit lamp you see that those capillaries become clouded. In a number of cases we have opened and closed those capillaries, where the patient is still under dietary control, by giving riboflavin or withdrawing it from the patient's diet.

The oral lesions are largely lesions of both lips in which there is a shiny denuded appearance and the lips appear to be chapped in many instances. The line of closure of the lips becomes quite red and very shiny and glistening. In exactly the corner of the lips appears a white spot which breaks down and you have a transverse fissure in each angle of the mouth, which extends on to the face, and we have never seen that extend into the mouth or to the cheek to any great extent. It may extend out to the face for a distance of quarter of an inch or half an inch. That is covered with a dry, yellowish secretion which you can pick off and you will see a raw, ulcerated crack underneath. It is exactly the picture described in the textbook as purulent.

In addition to these livid lesions in the mouth you have a discoloration of the tongue which has been described as being magenta color. The tongue is abnormal in that it has a purplish-red or magenta color which is different from the red tongue of nicotinic acid deficiency. The nicotinic acid deficiency is bright red. It does not have the purplish color that the riboflavin deficiency tongue has, and there is a change in the appearance of the papillae.

Lesions of riboflavin deficiency also appear in the eyes and in ulcerated lesions just inside the nares, where they are frequently seen. Occasionally we have seen them on the ears, in the form of an endomycosis involving both ears. I think they appear in the folds of the body. You might find them in the groin or under the arms. They are those typical greasy, yellowish, scaly accumulations which tend to pile up and have a mild erythematous lesion underneath them. In a few cases we have seen one or two that became secondarily affected with the streptococcus. The lesion has been described by Dr. Jolliffe and by the Duke workers as an accumulation of plugs in the glands across the nose and on the cheeks, which has been described as short skin.

DR. JOLLIFFE.—For many years we were calling this Pettingell's rash.

DR. SEBRELL.—They are hard plugs that fall out across the nose and on the cheek.

That covers the picture of riboflavin deficiency.

Those plugs are seen mixed up with the skin lesions of nicotinic acid deficiency, which is something else.

Dr. Jolliffe mentioned the dose of riboflavin per day as the dose he customarily used. That is a very reasonable dose, the one which we also use. Sometimes we can drop that down to 5 mg. However, 10 mg. is safer. The daily requirement of children is probably in the neighborhood of 2 mg., and of adults, between 2 and 3 mg.

In treatment I think 10 mg. is a good dose to use. From the administration of riboflavin there are no toxic symptoms that have ever been described. If you give larger doses or even in the doses that have been mentioned, a considerable amount is excreted in the urine, and when much larger doses are given, like 300 mg. a day, the excess simply pours out in the urine. It is all eliminated within four hours and there are no sores of any kind.

In using riboflavin you have to keep in mind that the material is light sensitive, especially when in solution. Putting a solution of riboflavin in a clear glass flask and setting it on the laboratory bench will result in rapid destruction of a considerable proportion. It does not require ultraviolet or strong sunlight. Its exposure to the ordinary light in the room tends to destroy the riboflavin.

In solution you should protect it from light. It is always better to protect the stuff from light, whether in solution or not. Keep it in a brown glass bottle and cover it with heavy brown paper. The solutions are always kept in flasks, in the same way. We keep those in a dark room. Do not leave any solutions of riboflavin standing around.

For the nicotinic acid deficiency, Dr. Jolliffe has told you of the encephalopathy which has recently been recognized. Before that we had always associated nicotinic acid deficiency with skin lesions. The lesions of nicotinic acid deficiency are oral lesions, gastrointestinal lesions, and skin lesions in addition to the encephalopathy and the mental changes. Most cases of pellagra have some degree of mental depression. Some of them become greatly depressed and show a tendency to commit suicide. A small proportion of them go on to a true dementia which can usually be cleared up.

I might extend a little bit the oral symptoms that I mentioned in connection with the riboflavin. In addition to the bright red tongue, you frequently find ulcers in the mouth. They may be on the tongue, under the tongue, or on the cheeks. It is usual to find associated with them a large number of buccal lesions which can be mistaken for Vincent's angina. An ulcerated mouth in which you get a smear of typical Vincent's organism may well be a nicotinic acid deficiency.

In black tongue in dogs we have ulcerations in the mouth. Those dogs invariably have large numbers of Vincent's organisms, which disappear as the mouth lesions clear up on the administration of nicotinic acid. The same is true of these human mouth lesions. The gastrointestinal lesions begin with indigestion and decrease as the amount of hydrochloric acid in the stomach becomes lessened. There are certain general symptoms. We have what we call heartburn or a pronounced sensation in the mouth.

CHAIRMAN TISDALL.—What is the difference between that and some of the symptoms of thiamine deficiency?

DR. SEBRELL.—They are the same, as far as I can find out. They are more apt to have a diarrhea with nicotinic acid deficiency. There is usually

a loss of weight and complaint of weakness. The patient usually calls it indigestion. The skin lesions always have been described as pellagra.

You must stop thinking of pellagra as a disease that is peculiar to the southern part of the United States. It is all around us, and frequently the diagnosis just is not made. The skin lesions, according to the textbook description, are bilateral and symmetrical. That is not always true, although sometimes they are. They usually begin very much like sunburn. You have the formation of large bullae, which rupture, and you may get a very disagreeable ulcerated surface. Sometimes those bullae do not form and the skin becomes intensely red, becomes dry and scaly, and these lesions are followed by pigmentation which may persist for years. I have seen a number of patients who had those things for ten years or longer following one attack of pellagra. They would customarily occur on the backs of the hands, on the dorsal area of the feet, and around the neck, although they may occur anywhere on the body. You may see them occur on the portions of the body which are most frequently exposed to sunshine and on the portions of the body usually subjected to trauma or rubbing, such as the elbows or the knees. It is common to find them in the vaginal region or in the scrotal region, and they may or may not be accompanied by lesions on the hands or face. We sometimes see dry, scaly lesions on the elbows and knees and, without the edema, on the hands. Practically always there is some skin lesion somewhere. You may have a butterfly lesion across the face or on the cheeks. I have seen a few on the forehead.

The question of the relationship of the skin lesions to sunshine is still question No. 1. It appears that anything that irritates the skin will intensify the skin lesions. It has been known since pellagra was first described that exposure to sunshine makes those skin lesions worse. I have frequently seen a patient sitting on the porch in rural sections, with the hands and arms in flour sacks or paper bags to keep the sunlight away from them, because they know it intensifies the lesions. You can make them worse by putting on an irritating ointment, by exposing them to a radiant heater, or anything which will make this dermatitis much worse.

We have talked about the therapeutic dosage of nicotinic acid. Do not forget to see that the patient has as adequate a diet as you can give him, because loss of appetite is a usual symptom. These patients have to be forced to eat.

In connection with the liver extracts I may add that the more purified these liver extracts are for pernicious anemia, which is the usual reason for producing the liver extract, the more purified and the higher the potency, the least valuable it is in these conditions. The cruder liver extracts seem to be the most effective in treating these conditions.

CHAIRMAN TISDALL.—I understand from this that you want us to make an exact diagnosis of whether the patient is suffering from riboflavin or nicotinic acid or thiamine deficiency and then give the proper dosage to cure the condition. Along with that you want us to make sure that the patient has an excellent diet and, if we are concerned with these other factors, we are to give some concentrate of the whole complex, perhaps a large dose of yeast. It is difficult with children to give a large amount.

Yeast concentrates are available on the market and I understand there will be some more, the potency of which will be very high. Then there are liver concentrates and there are some wheat germ concentrates containing all these members of the B complex.

In children suffering from a thiamine deficiency there is no use trying to overcome that by this complex which may give only one or two or three. It is better to give the pure thiamine; the same for the riboflavin and the same for the nicotinic acid.

Suppose that in his enthusiasm the pediatrician overdoes this 10 mg. dose of thiamine or 100 mg. of nicotinic acid, if we carry that along for a time and increase the dosage, is there any danger of a harmful effect?

DR. JOLLIFFE.—I question whether or not there would be any harmful effect. I have yet to see a patient develop toxic symptoms from thiamine. I have never seen any toxic symptoms from any of the other crystalline vitamins with, of course, the exception of nicotinic acid. However, there have been two cases reported of toxic reactions to the parenteral administration of thiamine. Those two cases seem to be perfectly well authenticated and reliable.

QUESTION.—With large doses of thiamine would it not be natural for a person to become allergic to that type of administration?

We have seen two subjects of whom referring doctors attributed symptoms to thiamine. The symptoms consisted of a feeling of itchiness and warmth and in one of them, in addition, a little difficulty in breathing as in asthma. In both of these patients, however, we could give crystalline thiamine dissolved in saline without symptoms. When we gave them the commercial preparation of thiamine, chlorobutanol as a preservative, symptoms developed. However, in the two patients reported in the literature, apparently the sensitivity was due to the thiamine and not to the preservative.

CHAIRMAN TISDALL.—In our pediatric practice infants suffering from vomiting are kept on a saline solution parenterally for some time. Do I understand that you are sold on the necessity of administering vitamin B to those patients?

DR. JOLLIFFE.—I am convinced of that because we can rapidly produce vitamin deficiency in our patients by putting them on glucose and saline solution. To compensate we give routinely a solution of 10 mg. of thiamine, 2 mg. of riboflavin, and 100 mg. of nicotinic acid each day that we give the infusion. In order partly to insure the unknown fractions which are impossible to "ampoulate," we also give about 5 c.c. of crude liver extract each day.

Too often we see patients with serious encephalopathic manifestations, due, we believe, to being given glucose without other essential elements of nutrition.

DR. SEBRELL.—One of the first things we observed was a very rapid decrease in the urinary elimination of riboflavin when it was withdrawn from the diet. A patient with riboflavin deficiency will be excreting daily in the urine less than 100 μg of riboflavin. A normal individual may be excreting anywhere from 700 to 1,500 or 1,600, depending on his diet.

If we take the normal individual excreting 600 or 700 and put him on a diet deficient in riboflavin, within seven or eight hours his excretion is equal to that of the patient who has riboflavin deficiency. Apparently the tissues hold on to such riboflavin as is present when riboflavin is withdrawn from the diet.

When you take a patient with diarrhea and vomiting who has been defrauded of vitamins and wash him out with saline and do not give him any food for two or three days, you have reduced that patient's riboflavin tissue content, and whether or not he develops symptoms is going to depend on how much he had before beginning the treatment, which is certainly a variable quantity.

CHAIRMAN TISDALL.—Tell us how frequently you think these symptoms occur among average children, say in this part of the country.

Is riboflavin deficiency found? Are we missing thiamine deficiency, riboflavin deficiency, and nicotinic acid deficiency in our everyday practice?

DR. SEBRELL.—I do not know how frequently it occurs in children. In adults riboflavin deficiency appears to be one of the commonest, if not the most common, deficiency disease in this country.

CHAIRMAN TISDALL.—Many pediatricians think of vitamin B deficiency in terms of just one thing, and that is thiamine. Many of the nutritional experts, such

as Dr. Sebrell, make the striking statement that riboflavin deficiency is more frequently encountered than thiamine deficiency.

DR. SEBRELL.—They found 245 cases in children in Birmingham in a two-year period. It was thought it was very common in the South. It may be more common there than it is here. Certainly all three of these things can occur together. I imagine the number of cases will depend on how thoroughly you look them over. I would be surprised if all of you did not find some in the poorer economic groups. Do not expect them to be limited to the poor, however. I have found some of these in rich individuals, in prominent individuals, and in some professors. You can expect to find them anywhere.

CHAIRMAN TISDALL.—Are there any special tests, either laboratory or otherwise, that we as pediatricians can make and that would be practicable in our office practice?

DR. JOLLIFFE.—That is a very hard question to answer if it were to be answered in any other way than "No."

The clinical examination can detect only the gross anatomic lesions. But if these gross signs were looked for, the apparent prevalence of nutritional disease would be multiplied many times.

The slit lamp is very valuable, but most of you do not have a slit lamp. The Lond slit lamp will not detect many of the minor corneal lesions that Dr. Sebrell has told you about, but you can see capillary invasion of the cornea when those capillaries are swollen with blood. This means that the deficiency is active. I do not believe we have a satisfactory chemical test for vitamin deficiency that can be used in routine practice, except, of course, blood ascorbic acid which will detect undersaturation of vitamin C.

DR. CASPARIS.—Can we depend today on a good diet supplying all those complex needs? Should we routinely add to the average diet certain vitamins pending better education?

CHAIRMAN TISDALL.—When we take the consumption statistics in Canada of white flour, sugar, and molasses, we find that the per capita consumption per day of these foods, on the basis of 2,500 calories, amounts to 53 per cent of the total calories consumed, and they are all extremely low or devoid of vitamins.

If you include fats, glucose, and corn syrup you will find that two-thirds of our national dietary contains practically no vitamins. That means that the whole burden of supplying these vitamins falls on just one-third of the food consumed.

So you see how you have to be so careful in planning your child's diet to see that that diet is made up of products that will supply vitamins. There is a real danger, I believe, unless the pediatrician keeps this in mind and does see that the diets are properly constituted. I would say that we do a better job than any other branch of medicine in seeing that our patients get the proper diet.

DR. CASPARIS.—You have too much confidence in our ability to secure a proper diet. Until we get these fundamental needs, it may be necessary to supplement them in many instances.

DR. SEBRELL.—The situation which Dr. Tisdall describes is not essentially different than the situation in this country. That was the reason which led us to attempt the introduction of the enriched flour and bread that you are hearing so much about. That was done with the advice of the National Research Council. It offers a means of meeting this difficult situation, because without the use of some

whole grain cereal or the use of this enriched flour in bread it is exceedingly difficult to get enough of the B complex in the diet to meet normal requirements in view of the increased use of highly refined foods. That is an attempt to put back in a white flour, which will be suitable for cakes, pies, and biscuits, the vitamins that have been taken away in the milling process, and the attempt to give them in a disguised way so that you can have your cake and eat it too. We have not been able to get Canadians to do that.

QUESTION.—You made the statement that vitamin B_1 was destroyed by heating. What becomes of it after the whole wheat is cooked into bread?

CHAIRMAN TISDALL.—There is about a 10 or 15 per cent vitamin destruction in the baking of bread. In toast you knock out quite a bit. In Melba toast you knock out 100 per cent.

QUESTION.—Did not Paul György show that the administration of one vitamin in a multiple vitamin deficiency produces a toxic reaction?

DR. SEBRELL.—That question apparently is based on some animal observations by György in California, indicating that with an experimental diet which is carefully restricted, following multiple deficiency, if you supply but one vitamin it appears to intensify the symptoms of the rest of the deficiency.

Whether that is of any practical importance in clinical work is problematic, because you do not have a patient with an experimental diet and you are attempting to give a full diet during your treatment. I do not believe that is of any particular clinical importance, although it has been observed in experimental animals under special conditions.

QUESTION.—Is vitamin B_1 or vitamin B complex of any value as a stimulant of a child's appetite?

DR. SEBRELL. It could not possibly be unless the child was lacking in the vitamin B fraction. In that case it probably would be.

QUESTION.—How much loss of vitamin B_1 is there in enriched bread?

DR. SEBRELL.—About the same as mentioned for the whole wheat bread. Depending on the method of baking the bread and the method of handling it, the loss is somewhere around 15 or 20 per cent, and the loss is below 10 or 15 per cent if you enrich it or dose it.

QUESTION.—Does the photophobin of acrodynia correspond to that spoken of today?

CHAIRMAN TISDALL.—There is no person who can answer that at the moment. It is worth while investigating children. The simplest and easiest way of recognizing riboflavin deficiency is to look at the eyes by means of a slit lamp. The earliest age at which you can examine a child with the slit lamp is 8 years. That eliminates infants.

QUESTION.—Are any of the members of vitamin B complex of value in the treatment of celiac disease and acrodynia?

DR. DRAKE.—There has been very extensive work done in Boston by May and McCreary in the treatment of celiac disease by the use of the vitamin B complex.

When they administer crude liver extracts parenterally, they get a dramatic response in this disease.

QUESTION.—What effect does irradiation of milk have on the riboflavin?

CHAIRMAN TISDALL.—Dr. Drake says that it has practically no effect at all.

DR. DRAKE.—On the other hand, if you leave a bottle of milk on the back steps in the morning sunshine for a few hours you will destroy all its riboflavin.

QUESTION.—What is your opinion as to radio and other advertising products containing one or more of the B complex elements?

DR. SEBRELL.—That is a question for the Federal Trade Commission which is charged with the enforcement of the law as to false and misleading statements. If you should see any advertising of these products that you think contains false or misleading statements, that is a matter for the Federal Trade Commission. The statements do not have to be false. They can be absolutely correct, but if they mislead the public such advertising is forbidden by the Federal Trade Commission. You write a complaint to them and they will take care of it.

QUESTION.—Should not the value of repeated small thiamine doses be stressed for building up depleted tissue reserves, since the extreme solubility of thiamine makes it rapidly excreted in the urine?

What about production of fatty livers in human beings when high thiamine doses are given?

DR. JOLLIFFE.—Thiamine, of course, should be given in divided doses.

As to the production of fatty livers in human beings by large thiamine doses, I do not believe that it has ever been observed. I do not believe we can go into the experimental work on this subject.

QUESTION.—What is inositol?

CHAIRMAN TISDALL.—Inositol is now regarded as one of the members of the B complex.

Book Reviews

Survey of Mentally and Physically Handicapped Children in Connecticut. A Report of the Committees on Education of Handicapped Children, Bulletin 23, Hartford, May, 1942, Connecticut State Department of Education, Division of Instruction, 95 pages.

The conclusions drawn from the study are striking and challenging. A reasonable list of recommendations should serve well for future action.

McCULLOCH.

Advances in Pediatrics. Adolph G. De Sanctis, M.D., editor, New York, 1942, Interscience Publishers, 306 pages. Price, $4.50.

This is the first of a series of annual reviews of pediatric advances. It is a collection of personalized monographs by selected authors. It is stated that such a publication will remedy the deficiencies created by the long intervals of several years that must or does exist between succeeding editions of textbooks. The material for this first volume was chosen by sending questionnaires to many pediatricians in this country requesting suggestions as to the real advances during the past two years.

The contents of the book, which could really be individual chapters, are as follows: toxoplasmosis, review of virus diseases, chemotherapy in diseases of infancy and childhood, electroencephalography, the role of vitamin K in hemorrhage in the newborn period, persistent ductus arteriosus and its surgical treatment, the premature infant, tuberculosis, endocrinology, and short abstracts of some other advances in pediatrics. Each of these is in excellent form and is not open to criticism. The uniformity of style and presentation has been well accomplished, and the material is well chosen and executed, thus overcoming the difficulties of assembling under one cover the writings of various authors.

Much can be said, however, for and against the future need for such an annual review of pediatrics. In one form or another reviews of pediatrics already exist in abundance, in other books, in loose leaf systems, in review journals, and as special articles in regular pediatrics journals. Certainly nothing new has been added by the appearance of this new review. There is also a question whether such a review will help the average physician keep abreast of pediatric literature. It is difficult to find the time to cover all the existing sources of literature, and it will never be possible by any means to keep the practicing physician fully read up to date. Whether one is abreast of the literature depends more on the habits of the individual who is trained to read and search out sources of information rather than on offering him more material to read.

The articles as printed will serve excellently as reference works over a period of several years so that cumulative indices at periods of five years would add to the value of the series. But by the end of that time the material would be found in a textbook. On examining the bibliography at the end of each article, there is a predominantly disappointing number of references which are recent enough to be included in a book of this type. These suggestions are directed not at the

book as it is, for it is excellent, but against the idea of adding more reading material to a field that is already replete. The future success of the series will determine the wisdom of the idea.

McCULLOCH.

Social Work Year Book, 1943. Russell H. Kurtz, Editor, Russell Sage Foundation, New York, 1943, ed. 7, 764 pages. Price, $3.25.

The present edition of the Social Work Year Book is the seventh to be published since the series was established in 1929. It undertakes, as did its predecessors, to report the current status of organized activities in social work and related fields. As each biennial issue is independent of the others, this volume may be consulted without the necessity of referring to earlier editions. It is presented as a concise, up-to-date encyclopedia in the area under review.

The book contains two major divisions: Part One, consisting of a group of seventy-eight signed articles written by authorities on the topics under discussion; and Part Two, consisting of four directories of agencies (national and state, both governmental and voluntary) whose programs are integral with or related to the subject matter of Part One. In addition, an extensive index has been supplied.

The topical articles are descriptive of functions, organized activities, and programs rather than of individual agencies. An attempt has been made to present a factual, cross-section view of organization and practice in the various fields as they appeared in 1942, with a minimum of historic background and of forecast. Important events occurring in the two-year period since the 1941 Social Work Year Book was published have been emphasized, particularly those resulting from the national defense and war developments. Coverage has been restricted to the United States except for one article, International Social Work.

The audience of this book should include not only social workers and practitioners in related fields, but also those in specialized fields, including medicine and pediatrics. It should be a standard reference work of the first order in the pediatric field.

McCULLOCH.

Social Work as a Profession. Esther Lucile Brown, Russell Sage Foundation, New York, 1942, ed. 4, 232 pages. Price, $1.

This monograph is one of a series dealing with the present status of certain established or emerging professions in this country. Four editions signify that there has been considerable interest in the presentation of this material and its usefulness.

In this edition the original form of presentation has been preserved, but almost all text, except the first few pages, has been rewritten. Change in social work during the 1930's was profound. This book more nearly completes the record of that change than was possible in 1938. Census data for the number of social workers in 1940 have recently become available and have been analyzed in some detail; so have additional figures for salaries for social workers. Educational facilities for the training of social workers are springing up throughout the United States. Much ferment is at work within the longer established schools and also within the American Association of Schools of Social Work. This recent growth and revitalization necessitated new evaluation of the purpose and achievement of professional education. Finally, some effort had to be made to interpret the present, and to foresee the possible future, effects of world cataclysm upon that profession which stands guard over an important sector of social welfare.

McCULLOCH.

Manual of Oxygen Therapy Techniques, Including Carbon Dioxide, Helium and Water Vapor. Albert H. Andrews, Jr., M.D., The Year Book Publishers, Inc., Chicago, 1943, 191 pages. Price, $1.75.

While it is stated that this manual is limited to technique, it is obvious that a great deal more information is included outside this field. The author describes in detail the correct administration of oxygen therapy and gives attention to many details which are frequently overlooked. He believes that proper dosage is basically a matter of achieving an effective oxygen concentration in the lungs. In order to achieve a given oxygen concentration in the lungs, it is important to be certain that a given oxygen flow is maintained under the best conditions of technique. These factors must be observed routinely to determine whether the therapy is being administered satisfactorily.

The gases described are oxygen, carbon dioxide, helium, and water vapor. The equipment for their administration is described, and the care of such equipment is discussed. Water vapor, not usually considered a therapeutic gas, has been included because the value of humidification is recognized more and more in the prophylaxis and treatment of certain diseases.

McCULLOCH.

Mental Illness: A Guide for the Family. Edith M. Stern, with the collaboration of Samuel W. Hamilton, M.D., The Commonwealth Fund, New York, 1942, 134 pages. Price, $1.00.

While this manual is devised as a guide book for the family and relatives, it is certain that it will prove useful to many other groups concerned with mental illness. The problem is not often presented to a pediatrician but frequently he is concerned in the structure of the family involved and may play an important part in its rehabilitation. The book is interestingly written, is easily readable, and represents a satisfactory contact with patients of this type. It can be recommended whole-heartedly as an accessory source of reading for any physician.

McCULLOCH.

Outline of Psychiatric Case Study. A Practical Handbook. Paul William Preu, M.D., Paul B. Hoeber, Inc., New York, 1943, ed. 2, 279 pages. Price, $2.75.

There have been extensive revisions in all sections of this edition following the use of the book in several clinics, especially at the New Haven Hospital. It is specially important that a separate portion of the book is devoted to the psychiatric case study of the child. This material covers somewhat less than one-half the total amount in the book. The material has been prepared in full detail so that it may be used entirely independently of the outline for use with the adult. It deals primarily with the problems of achievement of the school child and the adolescent. This limitation is deliberate and recognizes the fact that psychiatrists at the present time are working largely with older children and adolescents. It is the author's personal opinion that the problems of normal development and habit training in early childhood are, at the present time, dealt with more adequately by the specialists in child development or by the pediatrician with special training in the developmental field rather than by the psychiatrist. There is no discussion of the treatment, and the material is limited entirely to searching for abnormal mental states. It should be a useful guide to every pediatrician, even those who are not seeing older children. It is the author's belief that most of the problems of older children are the outgrowth of difficulties which are not adequately recognized and solved during early years.

McCULLOCH.

Human Embryology. Joseph Krafka, Jr., M.D., Ph.D., Paul B. Hoeber, Inc., New York, 1942, 395 pages. Price, $4.75.

One of a series of books designed specifically for the undergraduate student. This series is the outcome of a plan worked out by Dr. Fred C. Zapffe, Secretary of the Association of American Medical Colleges, with the assistance of others engaged in active teaching capacities, to provide a new kind of textbook which presents by sound teaching technique the basic foundation of the subjects. The books in no way resemble the old-fashioned "compendium" or outlines.

The books present in a rounded form the essential elements of the subject which the time allotted in today's curriculum permits the student to master. They are written in a style to encourage the student's inquiring instincts and to foster habits of further study. They offer the best of today's scientific concepts and methods of approach. The books are organized to present a closer correlation of the basic sciences with clinical medicine.

While these books are designed specifically for the undergraduate, they will, also, be helpful to the graduate who wishes to refresh his knowledge of the basic sciences. They will save him the time and trouble of digging material out of an encyclopedic tome.

This text of *Human Embryology* presents a short, complete account of the development of man. In order to reduce the amount of material, three topical matters of embryology have been greatly reduced. First, the reference to comparative embryology has been omitted; second, the elimination of virtually all reference to causal factors in development; and, third, the elimination of all references to laboratory exercises.

Illustrations are an indispensable necessity in any book of embryology. This problem has been met in a most desirable way by the use of a very large number of black and white line drawings. Many of these have been redrawn from illustrations from other sources. They are both clear and concise, presenting an understandable picture of a complex subject. The lack of color and absence of half-tone reproductions no doubt have reduced the cost of publishing the material without loss of usefulness of the illustrations.

McCULLOCH.

White House Conference on Children in a Democracy, Washington, D. C., January 18-20, 1940, Final Report. U. S. Department of Labor, Children's Bureau, Superintendent of Documents, Washington, D. C., 392 pages. Price, 65 cents.

This final report is issued by the Report Committee of the Conference pursuant to a resolution adopted by the conference at its final session and in accordance with instructions from its Planning Committee. The text was prepared for the Committee by its research staff after conferences with its officers. This report is a much fuller statement of facts and conditions about many phases of child life in America than space and time permitted in the more formal statements and recommendations based thereon, which were issued at the general conference in May, 1940. It could not be expected that the same unanimity and identity of point of view on the part of the Report Committee would be reflected in the present document of some 400 pages as was possible in an earlier report, each phase of which was considered, revised in many instances, and finally unanimously adopted by the entire conference in session in Washington in January, 1940.

The recommendations reproduced in Part IV are those which were approved and adopted by the Conference itself at its meeting on Jan. 19, 1940, and were published in the General Report of the Conference. The text of this report, as distinct from the recommendations, is based to a considerable extent on a series of special studies by the research staff covering the several fields of interest to the Conference.

The statements given above, taken from the foreword of this publication, give an unusually clear picture of the material and content of the Report, indicating its probable interest and usefulness in the field of pediatrics. One can compare this report with the ones from the three previous decennial White House Conferences especially the III Conference in 1930 to note the direction of interest in the welfare of children. The Conference in 1930 was in a large measure medical in its structure and, therefore, of unusual interest to pediatricians not only for the part played by them in the work and considerations of the Conference, but also for the information made available from the Conference. The publication from the III Conference still represents a highly important source of information and statement of basic pediatric principles, and the lapse of ten years has in no way diminished its value.

The IV Conference shows a shift of thought and action into the broader field of child welfare with still sufficient emphasis on child health and care to retain a strong pediatric interest. However, much of the material was collected and arranged before this country became involved in the present war, and many of the statements seem almost prophetic of the developments in the world at large today. This material should form a structure on which could be placed future developments in the next decade during the war period and, we hope, the postwar period.

McCULLOCH.